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Parasitoid-induced behavioral alterations of *Aedes aegypti* mosquito larvae infected with mermithid nematodes (Nematoda: Mermithidae)

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ABSTRACT: A wide range of parasites are known to cause behavioral changes in their hosts and parasitized insects are especially amenable to the study of such changes. The majority of studies addressing parasite-induced behavioral alterations have focused on parasites with complex life cycles and the adaptive nature of such changes. Behavioral changes caused by parasitoids, single-host parasites that kill their host upon emergence, have been studied less and the adaptive nature of these changes is likely to be different than those in complex life cycles. I investigated behavioral alterations in *Aedes aegypti* mosquito larvae infected with parasitoid nematodes (family Mermithidae). I conducted several experiments in which I tested the following hypotheses: 1) Mermithid nematodes induce behavioral changes in mosquito larvae and the changes are density dependent. 2) Different species of mermithid nematodes induce similar changes in mosquito larvae behavior. 3) Behavioral alterations vary with mermithid developmental stage. 4) Mosquito larvae infected with mermithid nematodes behave similarly to uninfected food-deprived mosquito larvae. I found that 4th instar *Ae. aegypti* infected with *Romanomermis culicivorax* or *Strelkovimermis spiculatus* exhibited resting behaviors significantly more often than uninfected controls but that intensity of infection did not affect activity levels. In earlier instars, infected mosquito larvae were more active than uninfected control larvae in some behaviors associated with feeding. There was no significant difference between infected and uninfected food-deprived mosquitoes in nine of the ten behaviors observed. The decrease in activity of late instar *Ae. aegypti* larvae infected with mermithids may be a parasitoid adaptation that reduces the risk of predation and thus increases host and parasitoid survival. The increase in feeding activity in earlier instars as well as the similarity between uninfected food-deprived and infected *Ae. aegypti* behavior may indicate that these behaviors are adaptive for the parasitoid, increasing nutritional acquisition for successful parasitoid development. *Journal of Vector Ecology* 31 (2): 344-354. 2006.

Keyword Index: Behavior, mosquito larvae, *Aedes aegypti*, host-parasite interactions, mermithid nematodes.

INTRODUCTION

Parasite-induced behavioral alterations in hosts are ubiquitous. Parasitized insects are especially amenable to the study of such changes, which may include alterations in behaviors as varied as thermal preference, mate choice, geotaxis, phototaxis, and predator avoidance. The majority of studies which investigate parasite-induced behavioral alterations in insect hosts have been conducted on parasites that have complex life cycles (see Moore 2002 for review). Fewer studies have addressed behavioral alterations induced by parasitoids. This relative lack of interest may stem from the assumption that because parasitoids kill their hosts, any behavioral change results from events associated with host death. In addition, many behavioral alterations in complex life cycles are thought to be adaptive for parasites by increasing predation risk (and therefore transmission) (Bethel and Holmes 1973, 1977, Moore 1983, 1984). Clearly, predation would not benefit parasitoids (but see Poulin 1992).

Nonetheless, when parasitoid-induced behavioral alterations have been examined, many of these studies have shown that even in the absence of complex life cycles,

behavioral changes may have adaptive consequences (Vinson and Iwantsch 1980, Stamp 1981, Fritz 1982, Slansky 1986, Brodeur and McNeil 1989, Schmid-Hempel and Müller 1991, Maeyama et al. 1994, Müller 1994, Vance 1996, Chow and Mackauer 1999). For instance, Stamp (1981) found that infected Baltimore checkerspot caterpillars infected with braconid wasp parasitoids selected different microhabitats (high and exposed) on vegetation than their uninfected counterparts. These high and exposed microhabitats might contribute to increased parasitoid survival by allowing the caterpillars to avoid hyperparasitism and by facilitating parasitoid mate-finding (Stamp 1981). Schmid-Hempel and Müller (1991) found that bumblebees infected with conopid flies spent more time outside the nest than did uninfected bumblebees, which may improve parasitoid dispersal and survival. Müller (1994) also showed that infected bumblebees dug into the soil before they died, thus providing the developing conopid parasitoid with a suitable hibernation site. Karban and English-Loeb (1997) showed that tachinid fly parasitoids altered food choice of caterpillar hosts. The change in food choice resulted in increased caterpillar survival, even after parasitoid emergence, and increased size of the parasitoid

(Karban and English-Loeb 1997). Fritz (1982) hypothesized that because parasitoid reproductive success depends upon host survival during parasitoid development, parasitoids should alter host behaviors in ways that reduce predation risk. Fritz (1982) further hypothesized that the type and extent of parasitoid-induced behavioral modifications should depend upon host life history traits and the timing and intensity of predation.

Based on the evidence that parasitoid-induced behavioral alterations can have adaptive significance, I investigated such behavioral alterations in *Ae. aegypti* mosquito larvae infected with mermithid nematodes. Nematodes in the family Mermithidae are parasitoids, some of which infect many species of mosquito larvae. *Romanomermis culicivorax*, the best studied mermithid species, is a mosquito host generalist, naturally infecting 17 species, including several species of *Aedes*, *Anopheles*, and *Culex*. An additional 78 species are susceptible when artificially exposed to this nematode in the laboratory or field (Petersen 1984, Blackmore et al. 1993). *Strelkovimermis spiculatus*, another generalist, has been found naturally in mosquitoes of the genera *Ochlerotatus* and *Culex* (Poinar and Camino 1986, García et al. 1994, Maciá et al. 1995, Becnel and Johnson 1998, Campos and Sy 2003, Achinelly et al. 2004). Both mermithid species infect *Aedes aegypti* mosquito larvae in the laboratory but have not been isolated from natural populations of that species. The life cycles of both *R. culicivorax* and *S. spiculatus* in mosquito larvae involve pre-parasitic non-feeding juveniles that hatch from eggs laid in the substratum of an aquatic environment. The pre-parasitic juveniles locate and burrow into early instar mosquitoes where they become parasitic and develop in synchrony with the mosquito larvae. The parasitic stage is the only stage in which the mermithid feeds. Over a period of six to eight days, the mermithid goes through several molts within the mosquito larvae; after this time, a post-parasitic nematode emerges, molts several more times, mates, then deposits eggs (Poinar 1983).

Mermithid-induced behavioral alterations have been studied in hosts other than mosquitoes. Maeyama and others (1994) found that ants infected with mermithid nematodes committed suicide by drowning. The authors hypothesized that the mermithids manipulated ant behavior in order to bring themselves to the ants as the appropriate habitat for nematode emergence. This change in host behavior resembles that induced by hairworms (Nematomorpha) on their orthopteran hosts (Thomas et al. 2002, Biron et al. 2005). In addition, Poulin and Latham (2002) found that beach hoppers infected with mermithid nematodes burrowed deeper than uninfected beach hoppers. Mermithid nematodes have also been shown to alter predator avoidance behaviors of their larval mayfly hosts (Benton and Pritchard 1990, Vance 1996, Vance and Peckarsky 1997, Williams et al. 2001). However, only one study (Vance 1996) supported Fritz's (1982) hypothesis that parasitoids alter behaviors in ways that reduce predation risk.

Although parasite-induced changes in adult mosquito feeding behavior have been and continue to be investigated,

especially in the realm of feeding (Koella and Packer 1996, Koella et al. 1998, 2002, Anderson et al. 1999, Hurd 2003, Ferguson and Read 2004), few studies have addressed parasite-induced behavioral alterations in larval mosquitoes (but see Welch 1960, Giblin and Platzer 1985, Webber et al. 1987a, 1987b). Mermithid studies mirror this imbalance. Behavioral changes induced by mermithid species that develop in adult mosquitoes have been investigated (Steiner 1924, Petersen et al. 1967, Blackmore 1994), with the majority of studies showing that mermithid parasitism rarely affects adult behavior (Petersen et al. 1967, Trpiš et al. 1968, Harlos et al. 1980, Ewing et al. 1989, Reardon and Lunt 1989, but see Doucet et al. 1979, Blackmore 1994). Behavioral studies of mosquito larvae infected with mermithid nematodes are less abundant (Welch 1960, Gaugler et al. 1984, Giblin and Platzer, 1985, Shamseldean and Platzer 1989). In one of the two studies that compared infected and uninfected larvae, Welch (1960) presented limited quantitative evidence that *Ae. aegypti* larvae infected with *Romanomermis neilsei* were half as active as uninfected larvae. Welch (1960) used the number of times the siphon of the mosquito larvae broke the surface of the water as an indicator of larval activity. The second study further indicated that mermithids affect larval behavior. Giblin and Platzer (1985) found that *Culex pipiens* and *Toxorhynchites amboinensis* infected with *R. culicivorax* had slower feeding rates than their uninfected counterparts. The study I present here is the third study to quantitatively assess the behavioral effects of mermithids on mosquito larvae, and the first to use multiple indicators (e.g. frequency and duration of more than five behaviors). I tested the following hypotheses: 1) Mermithid nematodes induce behavioral changes in mosquito larvae and these changes are intensity dependent. 2) Different mermithid species induce similar changes in mosquito larvae behavior. 3) Behavioral alterations vary with mermithid developmental stage. 4) Mosquito larvae infected with mermithid nematodes behave similarly to uninfected food-deprived mosquito larvae. I then discuss possible underlying mechanisms of the behavioral alterations and their potential adaptive significance.

MATERIALS AND METHODS

Mosquito rearing

A laboratory strain of *Ae. aegypti* mosquitoes established by Ballinger-Crabtree et al. (1992) from a wild population collected in Ogbomosho, Nigeria, was provided by Dr. William Black IV, Department of Microbiology, Immunology and Pathology, Colorado State University. The mosquitoes were reared and maintained under a 12:12 LD photoperiod at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Larvae were hatched under anoxic conditions in plastic shoeboxes (28 cm x 15 cm) filled with 2 liters of tap water (larval rearing containers). Upon hatching, 1st instar larvae were removed and placed into larval rearing containers at densities of 150-200 larvae/container. Larvae were fed ground Tetramin® Fish Food *ad libitum*. Pupae were removed and allowed to emerge

in adult rearing cages (150 pupae/adult rearing cage: 28 cm x 15 cm x 22 cm plastic box with a screen top). Adult mosquitoes were provided with sugar cubes and allowed to feed once a week on anesthetized mice. After the first blood meal an oviposition site was provided. Eggs laid on filter papers were collected twice between feedings and placed in plastic bags to be used for later experiments.

Infection procedures

Romanomermis culicivorax and *Strelkovimermis spiculatus* eggs were stored in plastic bags of moist sand at room temperature. Nematode hatching was initiated by flooding approximately 60 cm³ of egg/sand mixture with 100 ml of deionized water. At least 4 h later, the water was sampled for pre-parasitic nematodes and the number of nematodes per ml was calculated. First instar (n=200, <24 h of age) *Ae. aegypti* mosquito larvae were transferred into each of two 7 cm x 7 cm round plastic containers filled with 50 ml deionized water. Approximately 1,000 pre-parasitic nematodes were then added to one of the two containers (5:1 nematode:mosquito) and the total volume of deionized water in each container was brought up to 100 ml. Both groups of *Ae. aegypti* larvae (exposed to mermithids and unexposed) were held in these containers for 12-18 h without food. The infected larvae were then transferred into one larval rearing container and the uninfected larvae into another. Larvae were maintained in a manner similar to that used with stock colonies (see above). This procedure resulted in 50-60% infection prevalence with one to three mermithids per mosquito larva. Infection status was determined by observing mermithid nematodes through the cuticle of 4th instar mosquito larvae with the use of a dissecting microscope.

Experiment I – General behaviors

I tested whether infection and infection intensity with *R. culicivorax* or *S. spiculatus* influenced *Ae. aegypti* behavior. Four trials using *Ae. aegypti* mosquito larvae and *R. culicivorax* mermithid nematodes and five trials using *Ae. aegypti* larvae and *S. spiculatus* mermithid nematodes were conducted approximately 5 days post-infection. Each trial consisted of ten pairs of 4th instar *Ae. aegypti* mosquito larvae (one of each pair infected, one uninfected); each pair was isolated in a 4 cm x 4 cm plastic container with 40 ml tap water and a pinch of ground Tetra-min® fish food. Mosquito larvae were allowed to acclimate for 20 min. In order to reduce the influence of overhead light on larval behavior, a black cardboard square was placed above each container.

The behavior of a single *Ae. aegypti* larva in the pair was observed at eye level for 5 min; the remaining larva was observed thereafter. The observer did not know the infection status of the larvae because infection cannot be seen with the naked eye. Each behavior was recorded on a hand-held tape recorder in real time. If a behavior persisted for more than 5 s, it was recorded as another occurrence of the same behavior. The frequency of a single behavior

could later be assessed by counting the number of 5-s occurrences. A single observer recorded behaviors in all trials in order to maximize consistency. The behaviors that were recorded were modifications of those described by Walker and Merritt (1991; Table 1).

Immediately after both mosquito larvae had been observed, each was placed into a separate holding container for later dissection. Four to five h after observations, each larva was dissected and infection status determined. If a larva had pupated or died by the time of dissection, the behavioral data for that larva were not used. Data were analyzed using an analysis of variance split-plot model to account for trial, container, and mermithid species effects.

Experiment II – Behaviors during parasitoid-host development

I tested the hypothesis that infection with *R. culicivorax* affected the behavior of *Ae. aegypti* mosquito larva as the two developed (*Strelkovimermis spiculatus* mermithids were not tested as these colonies experienced mass mortality). First instar *Ae. aegypti* mosquitoes were infected with *R. culicivorax* as described above. Two trials were conducted at two, three, and four days post-infection (PI). Each trial consisted of ten pairs of *Ae. aegypti* larvae (one exposed/IN, one unexposed/UN). Thus, there were a total of six trials involving 120 mosquito larvae. At day four PI, infection status was determined by observing the presence or absence of a mermithid nematode through the larval cuticle. However, at days two and three PI, mermithid nematodes could not be seen through the cuticle; in these cases, IN larvae were selected randomly from exposed *Ae. aegypti* and were reared to 4th instars after the test to determine infection status. No mosquito was observed more than once and behavioral observations were carried out in a manner similar to that described in Experiment I with one exception. Prior to day four PI, mouthparts were too small to observe making US (underwater-still) and FS (suspension feed) indistinguishable. Therefore, a new combined designation, FS/US, was applied to motionless larvae when the movement of mouthparts was not visible (Table 1).

After both mosquito larvae had been observed, each was placed into a separate holding container for later dissection. Larvae observed prior to day four PI were allowed to develop until infection status could be determined. Larvae observed four days PI were dissected four to five h after the behavioral observations had been recorded, unless pupation or death intervened. In that case, behavioral data for that larva were not used. Data were analyzed using an analysis of variance split-plot model to account for trial, container, and day PI.

Experiment III – Food deprivation behaviors

In this set of experiments, I tested the hypothesis that the behavior of *Ae. aegypti* larvae infected with *R. culicivorax* mimicked that of uninfected food-deprived *Ae. aegypti*. In this way I could indirectly address nutrition as a possible

cause of parasite-induced behavioral alterations. Three trials were conducted using infected (IN), uninfected (UN), and uninfected food-deprived (FD) 4th instar *Ae. aegypti* mosquito larvae. Each trial consisted of ten observation containers, four with a pair of UN mosquitoes and six with a pair of IN and FD mosquitoes. UN and FD were not paired because they were indistinguishable. Infection procedures and the determination of infection status were carried out as described in Experiment I.

For each Experiment III trial, the establishment of the IN, UN, and FD groups of mosquitoes differed in the feeding regime and the day the mosquito eggs were set to hatch. Because depriving *Ae. aegypti* mosquito larvae of adequate food delays development (Nguyen et al. 2002; personal observation), mosquito larvae used in the FD group were hatched one day prior to those in the IN and UN groups, insuring that at the time of behavior observations, all mosquitoes would be in the same instar. In addition, measurements of the head capsule width of five mosquitoes from each treatment group were measured and compared against those by Christophers (1960) to confirm that they fell within the range typical of 4th instar *Ae. aegypti*. After the parasite exposure period, 100 larvae were removed from each group (IN, UN, and FD) and placed into larval rearing containers according to previously described methods (see Infection Procedures). UN and IN mosquitoes were fed Tetra-min® fish food *ad libitum*, whereas the FD mosquitoes were fed at a rate of 0.03, 0.04, 0.06, 0.06, 0.06 mg/larva/day for five days, respectively. In order to confirm that the feeding regime of the FD treatment group was sufficient to cause starvation, the total length of five mosquitoes from each treatment group were compared on the day of behavior observation. Behavior observations were carried out as described in Experiment I. An analysis of variance was performed to determine if there were significant behavioral differences among IN, UN, and FD mosquito larvae.

RESULTS

Experiment I – General behaviors

There was no trial or container effect; thus, data were analyzed treating both trial and container within trial as random variables. Infection with *R. culicivora* significantly affected bottom-feeding behaviors of *Ae. aegypti* larvae; IN larvae were significantly less likely to bottom feed than were UN larvae (Figure 1a). In contrast, infection with *S. spiculatus* affected several behaviors. IN larvae were significantly less likely to wriggle swim, suspension feed, bottom feed, chew substrate, and dive than were UN larvae (Figure 1b). However, UN larvae were significantly less likely to remain still (Figure 1b).

Although infection with *S. spiculatus* affected more mosquito larvae behaviors than infection with *R. culicivora*, larval behaviors did not differ significantly between the two infection treatments (Figure 1c). Therefore, data were pooled over mermithid species (IN = *R. culicivora* + *S. spiculatus*), increasing power and reducing type II errors.

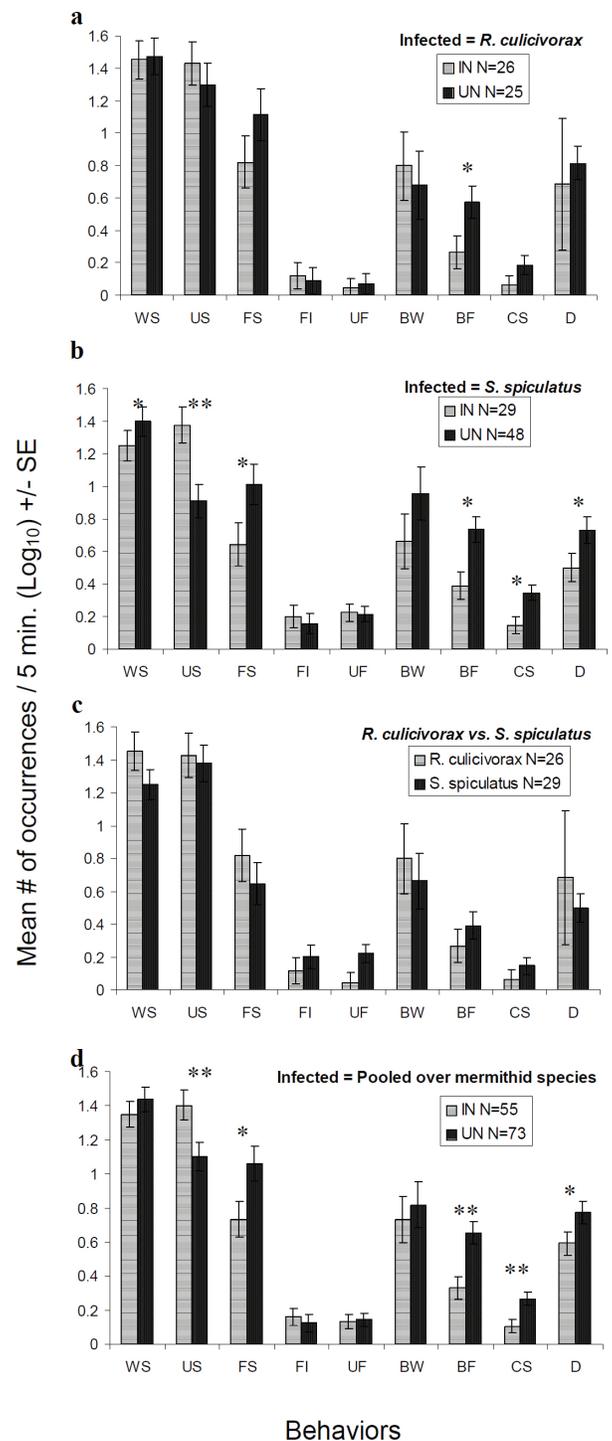


Figure 1. Mean number of occurrences (Log 10 transformed) of behaviors in infected and uninfected *Aedes aegypti* larvae over the course of 5 min (5 days PI). A) *Ae. aegypti* infected with *Romanomermis culicivora*, B) *Ae. aegypti* infected with *Strelkovimermis spiculatus*, C) Comparison between *Ae. aegypti* infected with *R. culicivora* and with *S. spiculatus*, D) Data combined over species. Mosquito behavior codes: WS, wriggle swim; US, underwater still; FS, suspension feed; FI, interfacial feed; UF, underwater suspension feed; BW, brush wall; BF, bottom feed; CS, chew substrate; D, dive. * = $P \leq 0.05$, ** = $P \leq 0.01$.

Table 1. Behaviors of *Aedes aegypti* mosquito larvae (adapted from Walker and Merritt 1991).

Behavior	Abbreviation	Description
<i>Wriggle-swim</i>	WS	Mosquito thrashes back and forth
<i>Still</i>	US	Still, mouthparts do not move
<i>Suspension Feed</i>	FS	Mosquito suspended by siphon from the surface of the container and mouthparts are moving. With or without movement of larva across surface
<i>Suspension Feed / Still</i>	FS/US	Either FS or US – larvae too small to determine mouthpart movement. Experiment II only.
<i>Interfacial Feed</i>	FI	Mosquito suspended by siphon from the surface of the container, body is bent with mouthparts brushing surface of the water
<i>Underwater Suspension Feed</i>	UF	Mosquito within the water column, mouth parts moving
<i>Brush Wall</i>	BW	Mosquito brushes wall of container with mouth parts
<i>Bottom Feed</i>	BF	Mosquito brushes bottom of container with mouth parts
<i>Chew Substrate</i>	CS	Mosquito manipulates food particles with mouth parts
<i>Dive</i>	D	Mosquito actively dives to bottom of the container

These data showed a significant behavioral difference between IN and UN larvae (Figure 1d). Suspension feeding, bottom feeding, chewing substrate, and diving all occurred significantly more often in UN larvae than in IN larvae (Figure 1d). IN larvae remained motionless significantly more than UN larvae (Figure 1d).

When asking if parasite intensity influenced behavior, I used only *R. culicivorax* because multiple infections of *S. spiculatus* were rare. I analyzed three different infection intensities: one mermithid, two mermithids, and three or more mermithids. There was no significance difference in behaviors among the infection intensities (Figure 2).

Experiment II – Behaviors during parasitoid-host development

On day two PI there was no significant difference in behaviors between IN and UN *Ae. aegypti* larvae with the exception of brushing wall (Figure 3a). On day three PI,

IN larvae exhibited significantly more wriggle swimming, bottom feeding, and diving behaviors than UN larvae (Figure 3b). On day four PI, IN larvae exhibited significantly more wriggle swimming and diving behaviors than UN larvae (Figure 3c).

Within each treatment (IN and UN), there were significant behavioral differences among developmental stages (Figures 4a and 4b). IN larvae on day two, PI exhibited significantly less interfacial feeding, wriggle swimming, still, suspension feeding, and diving behaviors than they did on days three, four, and five PI (Figure 4a). UN larvae were similar in that they exhibited significantly less still, suspension feeding, and diving behaviors on day two PI than on days three, four, and five PI. However, unlike their IN counterparts, they exhibited significantly more interfacial feeding than they did on days three, four, and five days PI (Figure 4b).

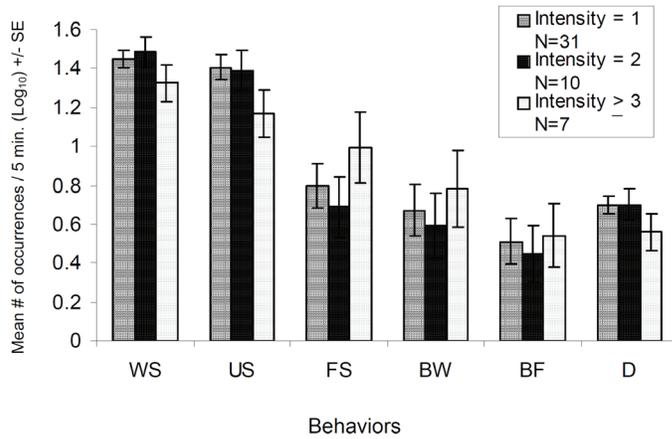


Figure 2. Mean number of occurrences (Log_{10}) of behaviors of *Ae. aegypti* infected with different intensities of *Romanomerms culicivorax* over the course of 5 min. Mosquito behavior codes: WS, wriggle swim; US, underwater still; FS, suspension feed; BW, brush wall; BF, bottom feed; D, dive.

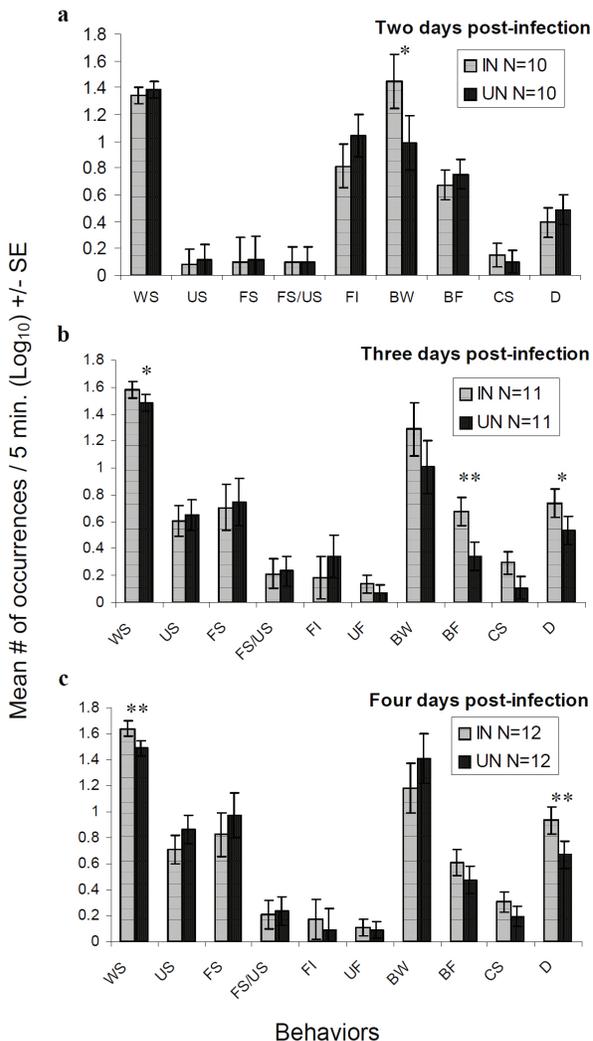


Figure 3. Mean number of occurrences (Log_{10}) of behaviors in uninfected *Ae. aegypti* mosquito larvae and those infected with *Romanomerms culicivorax* over the course of 5 min at 2, 3, and 4 days PI. Mosquito behavior codes: WS, wriggle swim; US, underwater still; FS, suspension feed; FS/US, suspension feed/still; FI, interfacial feed; UF, underwater suspension feed; BW, brush wall; BF, bottom feed; CS, chew substrate; D, dive. Within each behavior * = $P \leq 0.05$, ** = $P \leq 0.01$.

Experiment III – Food deprivation behaviors

The feeding regime for FD larvae was sufficient for inducing starvation. In trials one and three, FD larvae were significantly shorter (Trial 1: $n=5$, mean=2.9mm, range=3.5-4.5mm; Trial 3: $n=5$, mean=3.9mm, range=3.5-4.25mm) than IN (Trial 1: $n=5$, mean=5.75mm, range=5-6mm; Trial 3: $n=5$, mean=4.55, range=4-5mm) or UN larvae (Trial 1: $n=5$, mean=6.25mm, range=5.25-7mm; Trial 3: $n=5$, mean=4.75mm, range=4.5-5mm) ($P \leq 0.05$). In trial two, the mean total lengths of FD ($n=5$, mean=4.55mm, range=3.75-5mm) and UN larvae ($n=5$, mean=5.6mm, range=4-5mm) were not significantly different, but both were significantly shorter than IN larvae ($n=5$, mean=5.25, range=4.5-5.75mm) ($P \leq 0.05$). The head capsule widths of mosquitoes in all three treatment groups at the time of behavior trials fell within the range that characterizes late 3rd instar to early 4th instar larvae (UN: $n=15$, mean=0.83 mm, range = 0.55-1.0 mm; IN: $n=15$, mean=0.85 mm, range = 0.78-0.93 mm; FD: $n=15$, mean=0.82 mm, range = 0.55-0.93). According to Christophers (1960), late 3rd instar and early 4th instar *Ae. aegypti* head capsule width should be approximately 0.74 mm and 0.98 mm, respectively.

No significant differences were detected for wriggle swimming, interfacial feeding, brushing wall, and bottom feeding behaviors among the three treatment groups (ANOVA - $\alpha \leq 0.05$; Figure 5). In four of the five behaviors where there was a difference in frequency of the behavior (still, suspension feed, underwater suspension feed, and dive), UN differed from IN and FD larvae (Figure 5). IN and FD mosquito larvae exhibited significantly more still behaviors than did UN larvae (Figure 5). UN mosquito larvae exhibited significantly more suspension feeding behavior than either IN or FD larvae (Figure 5). The occurrence of underwater suspension feeding and chewing substrate was significantly higher in UN than in IN larvae, but was not significantly higher than in FD larvae (Figure 5). Diving occurred significantly more often in UN larvae than in FD larvae but not significantly more than IN larvae (Figure 5).

DISCUSSION

Aedes aegypti larvae infected (IN) with mermithid nematodes exhibited different behaviors than their uninfected counterparts (UN). Namely, IN larvae were more active in feeding and foraging behaviors early in mosquito and mermithid development and less active later in development than UN larvae. Also, IN larvae behaved similarly to UN larvae that had been food deprived (FD). Based on the pattern of these behavioral differences, the possibility exists that the behavioral alterations are parasitoid adaptations.

Poulin et al. (1994) indicated that the timing of parasite-induced behavioral alterations affects how parasites might benefit. They suggested that once infection occurred, the parasite could benefit by 1) immediately altering host behavior, 2) not altering host behavior, or 3) by altering host behaviors later in development. If an early

increase in feeding behavior sustains later development of both larvae and mermithid, then the parasitoid could benefit from reduced activity levels and reduced predation risk later in development. Evidence for adaptive stage-specific behavioral modifications has been shown in adult mosquitoes infected with *Plasmodium* (Koella et al. 2002). The results from this study may be an example of stage-specific behavioral alterations in larvae.

Early in development IN mosquito larvae brushed wall, bottom fed, dived, and wriggled significantly more than UN larvae. These results are different from those by Giblin and Platzer (1985) who showed that feeding rate of infected *Cx. pipiens* was slower and was not affected by developmental stage. Despite Giblin and Platzer's (1985) conclusions, the increase in feeding and foraging behaviors of IN larvae in this study is consistent with the hypothesis that parasitoids, which rely on host nutrition for development, should alter host behaviors in ways that facilitate their development (Thompson 1990). Furthermore, because the mosquito host does not become nutritionally stressed by the presence of mermithid nematodes until four days PI (Gordon et al. 1974), it is possible that by increasing foraging early in development, mermithids allow their host to acquire sufficient nutrients and thus develop adequate fat body to support later development. Interestingly, the study by Giblin and Platzer (1985) seems to support this idea. They found that despite the slower feeding rate, parasitized *Cx. pipiens* larvae had higher gross conversion efficiencies (GCE) at days one through four PI than did uninfected larvae. They hypothesized that the higher GCE of parasitized larvae early in infection could be a result of conservation of energy due to decreased movement or of increased efficiency of nutrient utilization, digestion, and/or absorption. Based on my results that early in development IN *Ae. aegypti* larvae are more active than UN larvae, the latter hypothesis has more support. If a gain in food stores, or the ability to have increased nutrient utilization does occur in infected larvae early in development, then this may enable the parasitoid to reduce host activity levels and thus predation risk later in development when predation risk is higher (Fincke et al. 1997). Meanwhile, behaviors of IN and UN larvae track each other as they develop, signifying that mosquito development itself also influences behavior.

Later in development (day five PI) IN mosquito larvae spent significantly more time motionless and significantly less time filter feeding, bottom feeding, chewing substrate, and diving than did UN larvae. These results are consistent with both Welch's (1960) and Giblin and Platzer's (1985) conclusions that mosquito activity is hindered by mermithid infection. The behavioral alterations were not influenced by parasite intensity. However, because high intensity infections (≥ 3 nematodes/mosquito) were rare, it is possible that a type II error may have occurred. The shift in behavior from IN being more active to less active than UN larvae later in development supports my earlier hypothesis that a gain in food stores early in development may enable parasitoids to reduce host activity later in development. Furthermore, because activity of mosquito

larvae is correlated with predation rate (Sih 1986, Juliano and Reminger 1992, Yasuda and Mitsui 1992, Grill and Juliano 1996, Juliano and Gravel 2002, Kesavaraju and Juliano 2004), Fritz's (1982) hypothesis that parasitoids should alter behaviors of their host in ways that decrease the risk of predation is consistent with these data.

Although behavioral modifications of *Ae. aegypti* by mermithid nematodes could be adaptive (increased foraging to meet nutritional demands or avoidance of predators), the possibility exists that changes in host behavior may be a side effect of a lethal infection with no adaptive value, especially if the behavioral alterations occur close to the time of host death. However, this explanation is contradicted by the fact that pathological effects on host behavior can also be considered a mechanism of adaptation if they occur routinely and have adaptive value (Moore 2002). Holmes and Zohar (1990) identified several pathological effects of infection that could result in changes in host behavior: organ malfunction due to direct tissue damage, modulation of endocrine control systems, and impaired nutrition. Of these, impaired nutrition is the most likely candidate for a mechanism, both because direct tissue damage and modification of the endocrine control system do not occur in this host-parasitoid system (Gordon 1981, Poinar 1983) and because mermithid nematodes, like many other parasitoids, place nutritional demands on their hosts (Bailey and Gordon 1973, Gordon, 1981, Gordon et al. 1981). The fact that *Aedes vexans* infected with *R. culicivora* resemble starved uninfected larvae in their development, growth, and failure to develop significant fat body (Galloway and Brust 1985) provides further evidence for a nutritional explanation for the behavioral changes reported here.

By comparing IN larvae with food-deprived and well-fed UN larvae I tested whether impaired nutrition played a role behavioral changes. There was a significant difference in five of the nine behaviors among the three treatment

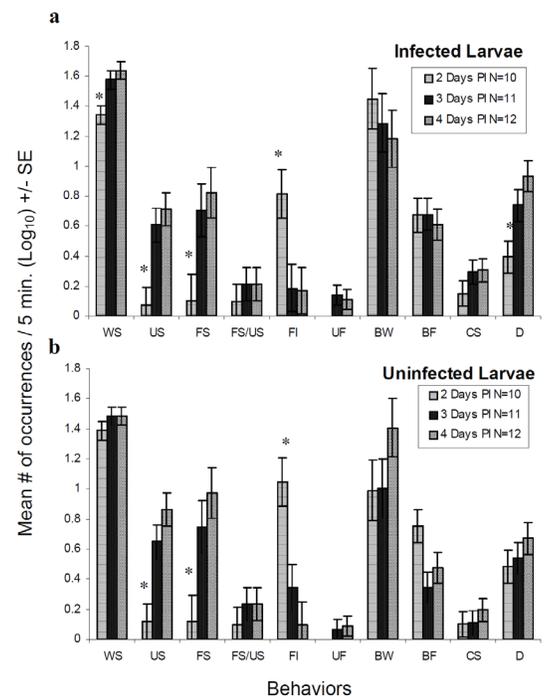


Figure 4. Mean number of occurrences (Log₁₀) of behaviors in *Ae. aegypti* mosquito larvae a) infected with *Romanomeris culicivora* and b) uninfected *Ae. aegypti* mosquito larvae over the course of 5 min at 2, 3, and 4 days post-infection. Mosquito behavior codes are: WS, wriggle swim; US, underwater still; FS, suspension feed; FS/US, suspension feed/still; FI, interfacial feed; UF, underwater suspension feed; BW, brush wall; BF, bottom feed; CS, chew substrate; D, dive. Within each behavior, * = P < 0.01.

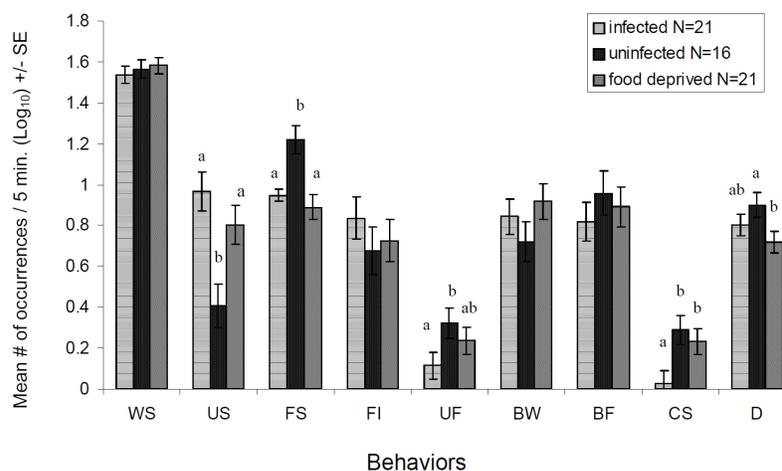


Figure 5. Mean number of occurrences (Log₁₀) of behaviors of *Ae. aegypti* infected with *Romanomeris culicivora*, uninfected *Ae. aegypti*, and food deprived uninfected *Ae. aegypti* over the course of 5 min. Mosquito behavior codes are: WS, wriggle swim; US, underwater still; FS, suspension feed; FI, interfacial feed; UF, underwater suspension feed; BW, brush wall; BF, bottom feed; CS, chew substrate; D, dive. Within each behavior, means sharing the same letter are not significantly different (P ≤ 0.05).

groups. In four of the five behaviors (still, suspension feed, underwater suspension feed, and dive), IN and FD were not significantly different. Specifically, both IN and FD larvae exhibited significantly more still behaviors and significantly fewer suspension feeding behaviors than UN larvae. Only one of the two treatment groups differed significantly from UN in suspension feeding and diving. Although no significant difference in the occurrences of wriggle swimming, interfacial feeding, brushing wall, and bottom feeding behaviors among the three treatment groups was detected, IN and FD larvae appear to be more similar than either are to UN. These data support the hypothesis that impaired nutrition due to mermithid parasitism may be the underlying mechanism for behavioral modifications. Clearly then, pathology from food deprivation may result in altered behavior as a way to avoid predation risk or may be an unavoidable correlate of mermithid demands on growth.

The result of this study is the observation that mosquito larval activity is modified by mermithid infection. The nature of the modifications differs depending on mosquito developmental stage. For early instars, there was an increase in feeding activity, whereas later instars exhibited a decrease in activity compared to uninfected control. The stage-specific nature of these behavioral alterations may indicate that they are parasitoid adaptations that increase parasitoid survival. An increase in feeding early in development may provide the necessary nutrients needed for successful development of the mermithid and the decrease in activity later in development could enhance the fitness of mermithids by decreasing predation on their mosquito hosts. Further studies that address the nutrition status of infected and uninfected mosquito larvae and those that incorporate a predator are needed before one can assess whether mermithid-induced behavioral alterations are indeed parasitoid adaptations that increase parasitoid survival.

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