

## Scientific Note

**Predator avoidance behavior of *Aedes aegypti* mosquito larvae infected with mermithid nematodes (Nematoda: Mermithidae)**Megan R. Wise de Valdez<sup>1</sup>*Department of Biology, Colorado State University, Fort Collins, CO 80523-1878, U.S.A.**Received 11 September 2006; Accepted 21 February 2007*

Parasite-induced behavioral alterations in hosts are ubiquitous and many studies have suggested those changes are parasite adaptations to increase parasite transmission and survival (see Moore 2002 for review). The majority of studies have been conducted on parasites with complex life cycles; however, several 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, Brodeur and McNeil 1989, Schmid-Hempel and Müller 1991, Maeyama et al. 1994, Müller 1994, Vance 1996, Chow and Mackauer 1999). Parasitoids are an example of parasites with a direct life cycle and they usually kill their hosts upon emergence. 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.

Nematodes in the family Mermithidae are parasitoids, some of which infect many species of mosquito larvae. *Romanomermis culicivorax* and *Strelkovimermis spiculatus* are both mosquito host generalists and have been found naturally in several mosquito genera, including several species of *Aedes* (Petersen and Chapman 1979, Poinar and Camino 1986, Maciá et al. 1995). Both species infect *Aedes aegypti* mosquito larvae in the laboratory but have not been found in natural populations of that species. The life cycle of *R. culicivorax* and *S. spiculatus* involves three main stages: egg, parasitic juvenile, and free-living adult. The parasitic juveniles locate early instar mosquito larvae and burrow into the haemocoel where they develop in synchrony with the mosquito larvae. After a maturation period that lasts six to eight days, they emerge from their hosts.

Mermithid-induced behavioral alterations have been studied in non-mosquito hosts (Benton and Pritchard 1990, Maeyama et al. 1994, Vance 1996, Vance and Peckarsky 1997, Williams et al. 2001, Poulin and Latham 2002), but only Vance's study (1996) supported Fritz's (1982) hypothesis when she showed that infected mayfly larvae drifted less than uninfected mayfly larvae and thus reduced their

exposure to fish predators. Several studies have shown that mermithid-induced changes in activity levels of mosquito larvae occur, but none have incorporated predators (Welch 1960, Giblin and Platzer 1985, Wise de Valdez 2006). Because activity levels of mosquito larvae directly impact predation (Sih 1986, Yasuda and Mitsui 1992, Grill and Juliano 1996, Juliano and Gravel 2002), activity differences between infected and uninfected *Ae. aegypti* larvae may influence predation risk by a natural predator, *Tx. rutilus*, a sit-and-wait ambush predator.

Therefore, following Fritz (1982), I predicted that in response to an alarm stimulus or predator, mermithid nematodes would exhibit activity levels lower than those of uninfected larvae. Lower activity should reduce predation risk and lead to increased parasitoid survival. It may be argued that this is a flawed hypothesis because this particular parasite-host system does not occur in nature and therefore parasite manipulation of host behavior could not have evolved. However, because *R. culicivorax* and *S. spiculatus* are mosquito host generalists and have been found naturally in other *Aedes* species that share traits with *Ae. aegypti*, it is conceivable that a manipulative effort could have evolved in these hosts and then be manifested in *Ae. aegypti*. The fact that some acanthocephalan parasites of birds that alter behaviors of their native intermediate host (*Gammarus pulex*) also change those of a recent invader (*Gammarus roeseli*) indicates that manipulation of a novel congeneric host is possible (Bauer et al. 2000, 2005). In addition, depending on the mechanism involved in behavioral change, we expect related hosts to be more likely to share those mechanisms and if they are shared, a parasite could use that mechanism even in a novel host.

A laboratory strain of *Ae. aegypti* mosquitoes was reared and maintained as per Wise de Valdez (2006). *Toxorhynchites rutilus rutilus* were maintained in a 12:12 LD photoperiod at 27° C ± 2° C; individual larvae were reared in 4 cm x 4 cm open-topped plastic specimen jars filled with tap water. They were fed a daily ration of ten *Ae. aegypti* larvae of the same instar as the developing *Tx. rutilus*. Infection of *Ae. aegypti* with mermithid nematodes was initiated seven days prior to behavioral experiments and is outlined in Wise de Valdez (2006).

In the first experiment, I tested whether mermithid infection affected the response of 4<sup>th</sup> instar *Ae. aegypti* mosquito larvae to an artificial alarm stimulus. Three trials were conducted and each involved three 10 cm x 10 cm

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containers filled with tap water, a pinch of ground Tetra-min® fish food, and either ten infected (IN: *R. culicivora*x or *S. spiculatus*) or ten uninfected (UN) 4<sup>th</sup> instar *Ae. aegypti* larvae. An alarm stimulus, a black cardboard square attached to a wand and passed over each container, was applied after all larvae were present at the surface. The time (s) that it took for half of the mosquitoes to return to the surface after diving was recorded. The stimulus was applied four times at 15-min intervals. Recovery times were log-transformed and a two-way analysis of variance was performed using Proc Glm in SAS® version 9.1 to determine if there were trial or replication (15-min repeat of stimulus) effects; these were not significant and therefore were treated as random in the subsequent analysis of variance using Proc Mixed in SAS® version 9.1. Larvae infected with *S. spiculatus* took significantly longer to recover after the alarm stimulus than did UN larvae ( $P = 0.0265$ ). Recovery times between the two IN groups as well as between larvae infected with *R. culicivora*x and UN larvae were not significantly different ( $P > 0.05 =$  not significant).

In the second experiment, I tested the effect of *Tx. rutilus* on IN *Ae. aegypti* larval behavior. Only *R. culicivora*x was used due to mortality in *S. spiculatus* colonies. Four trials were conducted. Each trial consisted of two treatment groups: 1) Predator Present (PP): five 10 cm x 10 cm plastic containers, each with 100 ml conditioned water (water

in which one *Tx. rutilus* larva had been feeding for ten days), one well-fed *Tx. rutilus* larva, one IN and one UN 4<sup>th</sup> instar *Ae. aegypti* larva. 2) No Predator (NP): five 10 cm x 10 cm plastic containers, each container with 100 ml tap water, one IN and one UN 4<sup>th</sup> instar *Ae. aegypti* larva. A total of 20 IN and 20 UN larvae were observed in each of the two treatment groups (PP and NP) over the course of four trials. Behavioral observations were carried out as per Wise de Valdez (2006). For analysis of behaviors, I combined recorded behaviors (Wise de Valdez 2006) into three risk categories: low, moderate, and high. Behaviors that brought *Ae. aegypti* larvae into direct contact with *Tx. rutilus* or those that attracted the attention of *Tx. rutilus* were considered high risk. Moderate risk behaviors were those where there was a potential for predator contact and a moderate activity level. Resting and other low-activity behaviors near the top of a container were considered low-risk behaviors. The number of occurrences of each behavior was log transformed and data were analyzed using a two-factor analysis of variance of a split-plot model using Proc Glm in SAS® 9.1 to determine if there was a trial effect; there was none. Therefore, the two-factor analysis of variance of a split-plot model was analyzed using Proc Mixed in SAS® version 9.1 and treating trial as random. There were no significant differences in behaviors between IN and UN larvae in both the NP and PP treatments (Figure 1). The

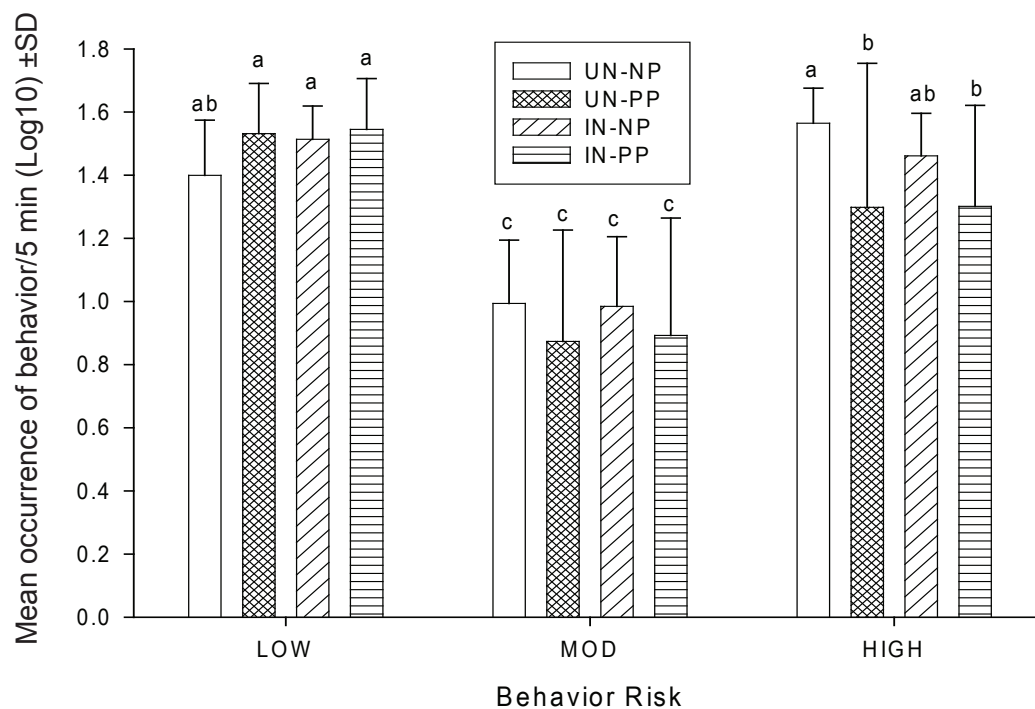


Figure 1. Mean number of occurrences ( $\text{Log}_{10}$  transformed  $\pm$  SE) of low, moderate, and high risk behaviors of 4<sup>th</sup> instar *Aedes aegypti* mosquito larvae infected with *Romanormis culicivora* (IN) or uninfected (UN) with (PP) and without (NP) the presence of the predator *Toxorhynchites rutilus*. UN-NP n=14, UN-PP n=22, IN-NP n=15, IN-PP n=23. Means with the same letter are not significantly different ( $P$ -values  $> 0.05 =$  non-significant).

presence of *Tx. rutilus* caused both IN and UN *Ae. aegypti* larvae to shift to significantly more low-risk behaviors than high-risk behaviors. However, within the high-risk behavior classification, only UN larvae significantly decreased these behaviors when presented with a predator (Figure 1).

In the third experiment, I tested whether *Tx. rutilus* preys more readily on IN or UN *Ae. aegypti* when given the choice. The experiment consisted of five trials. Four trials had three replicates and one trial had two. Each replicate consisted of one *Tx. rutilus*, which had been deprived of food for 24 h, and 20 4<sup>th</sup> instar *Ae. aegypti* larvae (10 IN and 10 UN) in a 10 cm x 10 cm container of conditioned water. The number of *Ae. aegypti* larvae was recorded at 3-h intervals until 50% of the mosquitoes remained. Any *Ae. aegypti* larva that died naturally or pupated was removed and not included in the analysis. A chi-squared analysis and Fisher's exact test were performed. In four of five trials, infection status did not significantly affect consumption. When combined over all trials, the odds ratio indicated that IN larvae were 0.78 times less likely to be consumed by *Tx. rutilus* than UN larvae and this was not significant ( $P = 0.46$ ).

In the first experiment, larvae infected with *S. spiculatus* reduced their activity levels beyond those of UN larvae; however this result may reflect nutritional differences rather than a direct manipulation of anti-predator behaviors by mermithid nematodes. Olsson and Klöwden (1998) found that nutritionally deprived *Ae. aegypti* larvae dived less in response to an artificial alarm stimulus and stayed below the surface longer when they did dive than did well-fed controls, suggesting that because diving is energetically expensive, food-deprived larvae conserve energy by modifying their behavior. Mermithid nematodes place nutritional demands on their mosquito hosts (Gordon 1981) and mosquitoes infected with mermithids behave similarly to food-deprived uninfected larvae (Wise de Valdez 2006). Therefore, it is likely that IN larvae also modify their behavior to conserve energy. This explanation gains support from the fact that these behavioral differences did not translate into a difference in predator avoidance success. However, this may also be explained by the predator behavior of *Tx. rutilus*, which attacks from below rather than from above as was modeled by the artificial alarm stimulus. Further studies using a predator that attacks from above would help to resolve this question.

The results from the second experiment in which infection status did not affect the overall predator avoidance behaviors appear to support the conclusion that changes in behavior may be due to nutritional differences between IN and UN larvae. The fact that only UN larvae significantly reduced high risk behavior in the presence of a predator indicates that IN larvae already exhibited lower levels of activity prior to the addition of a predator. These results are consistent with Wise de Valdez (2006) who showed that 4<sup>th</sup> instar *Ae. aegypti* larvae infected with either *R. culicivora* or *S. spiculatus* exhibited significantly more resting behaviors than did UN *Ae. aegypti*. It is likely that the low activity levels are a form of energy conservation due

to nutritional depletion rather than direct manipulation of predator avoidance behaviors by the nematode because these behaviors occur regardless of the presence of a predator. In addition, the fact that mermithid nematodes did not reduce activity levels below those of UN controls in the presence of a predator suggests that there may be a threshold of decreased activity beyond which mosquito larvae, regardless of infection, cannot go (Moore 2002). It is also possible that mermithid nematodes and their hosts face a trade-off between limiting high-risk behaviors to reduce predation risk and maintaining those feeding behaviors necessary for host and parasitoid development (Benton and Pritchard 1990, Vance 1996).

Mermithid infection did not alter predation rate on *Ae. aegypti* larvae by *Tx. rutilus*. In fact, predation on both treatment groups of *Ae. aegypti* appeared to be unpredictable with more UN larvae consumed in two of five trials. Because differences in predation rates on mosquito species are often due to differences in predator avoidance behaviors (Sih 1986, Yasuda and Mitsui 1992, Grill and Juliano 1996), the absence of differential predation on IN and UN *Ae. aegypti* by *Tx. rutilus* is consistent with the absence of differences in predator avoidance behaviors between the two groups in experiment 2.

My prediction, that in response to an alarm stimulus or predator, mermithid nematodes would reduce activity levels of larvae beyond those of UN larvae and that these changes in behavior would reduce predation risk leading to increased survival of the parasitoid, was not supported by the data. Therefore, in this system mermithid-induced behavioral alterations are not parasitoid adaptations to increase survival.

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