RESEARCH NOTES

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Moniliformis moniliformis Infection Has No Effect on Some Behaviors of the Cockroach *Diploptera punctata*

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ABSTRACT: The behavior of the cockroach *Diploptera punctata* parasitized with the acanthocephalan *Moniliformis moniliformis* was examined for parasite-induced alterations. No significant difference in behavior was found between parasitized and unparasitized animals in the following behavioral tests: (1) choice of white/black, horizontal/vertical substrate under light and dark conditions; (2) temporal and directional response to a bright light source; (3) choice between light and dark (photophilia); and (4) activity (time spent moving, distance, and velocity). A comparison of uninfected animals under 2 light conditions showed that light affected the activity of uninfected animals and their response to substrate. *Diploptera punctata* is the first nondomestic cockroach to be examined for behavioral responses to *Moniliformis* infection. This is the first report of an arthropod in which acanthocephalan infection has failed to alter behavior under at least some common test conditions.

The ability of acanthocephalans to alter arthropod intermediate host behavior is widespread (Holmes and Bethel, 1972; Moore and Gotelli, 1990). In a series of laboratory experiments (Carmichael and Moore, 1991; Gotelli and Moore, 1992), the behavioral responses of several cockroach host species to acanthocephalan (*Moniliformis moniliformis*) infection have been examined. In this study, we report the results of such experiments with *Diploptera punctata* (=*Diploptera dytiscoides*), a viviparous, widespread native of the South Pacific (Woodhead, 1984).

Maintenance of cockroaches, exposure to parasites, and general experimental protocol followed the methods of Carmichael and Moore (1991). Female cockroaches were not used because of potential behavioral variation associated with reproduction (Lipton and Sutherland, 1970).

Acanthocephalan eggs were obtained from stock originating in wild rats in the Houston Zoological Gardens. Cystacanths from *D. punctata* were fed to 2 4-mo-old white rats. Nine weeks later, 1 of these rats harbored *M. moniliformis* adults (4 gravid females and 1 male). Thus, the life cycle of *M. moniliformis* was completed successfully using *D. punctata* as an intermediate host.

Experiments were conducted under 2 light conditions: white light and red light (Carmichael and Moore, 1991). The latter is thought to be outside the cockroach visible spectrum (Seelinger and Tobin, 1982), allowing roach observation in simulated darkness.

Substrate choice under both red and white light was examined in an arena in which one-half of the floor and adjacent wall was black, the other half was white. The response variable was the fraction of time spent on each of the 4 possible combinations of color (black, white) and orientation (horizontal, vertical).

The activity test involved a grid covering the floor of a white arena (1 square = 5 cm^2, designated with Cartesian coordinates). Insect location was recorded, with vertical positions noted as the nearest horizontal square. From this we determined the total number of movements, the total distance moved, and average velocity (cm/sec) during periods of movement.

Phototaxis was examined in a white arena under red light. A bright white light was placed above 1 point along the arena perimeter. Each animal was acclimated in the center of the arena under an inverted opaque cup (5 cm diameter). The light was then turned on and the cup lifted. Freeze time equaled the time between lifting the cup and the first major movement of the animal, excluding cleaning behavior or turning in place, and represented the response time of the animal to the light. First direction of movement was scored on a scale of 0 (directly toward the light) to 6 (180° away from the light).

Photophilia tests involved a black arena under white light, with 1 side covered by a black semicircular sheet of Lexan approximately 15 cm above the floor of the arena. The response variable was roach location (light or dark portion of the arena).

These experiments were designed to assess simultaneous effects of light and parasitism on *D. punctata* behavior. Data were analyzed using a repeated measures ANOVA with the within-subjects factor being light (red or white) and be-

TABLE I. Behavioral scores for uninfected (n = 40) and infected (n = 35) *Diploptera punctata* (mean ± SD).

	Unifected		Infected	
Freeze time (sec)	31.0 ± 48.1		36.8 ± 44.7	
% Time in light	33.8 ± 21.1		36.2 ± 24.9	
	Red light	White light	Red light	White light
Substrate choice (% time)				
Black horizontal	30.6 ± 20.4	39.0 ± 36.6	28.4 ± 20.0	48.9 ± 39.8
Black vertical	32.7 ± 24.5	38.9 ± 38.8	32.5 ± 28.5	31.6 ± 41.4
White horizontal	21.9 ± 15.9	10.4 ± 18.8	24.4 ± 16.7	11.6 ± 20.7
White vertical	18.5 ± 19.4	11.7 ± 24.9	14.7 ± 19.2	8.4 ± 22.1
Activity				
% Time inactive	14.5 ± 18.0	73.2 ± 28.8	17.1 ± 22.4	68.7 ± 32.6
Total distance (cm)	505 ± 615	1,714 ± 662	613 ± 745	1,625 ± 750
Velocity (cm/sec)	2.3 ± 1.11	2.22 ± 0.60	2.03 ± 0.76	2.14 ± 0.71

tween-subjects factor being parasitism (parasitized or unparasitized). Unless otherwise noted, sample sizes were 40 uninfected and 35 infected animals. Percentages, distances and velocities, and freeze times were arcsine square root-, log-, and square root-transformed, respectively. A chi-square test was performed on phototaxis (direction) data from 26 infected animals and 25 uninfected animals.

Results are summarized in Table I. The percentage of time spent on 4 possible substrates under red or white light did not differ between infected and uninfected animals, nor did the percentage of time active, total distance moved, or average velocity ($P > 0.10$). Infected and uninfected *D. punctata* did not differ in directional ($\chi^2 = 5.392$; df = 6; $P = 0.49$) or freeze time (mean square = 0.012; $F = 0.17$; $P = 0.68$) responses to a bright light. There was no significant difference between infected and uninfected *D. punctata* in the percentage of time spent in light (mean square = 0.005; $F = 0.06$; $P = 0.80$).

Although parasitism produced no significant behavioral effect, uninfected animals were influenced by light. Under white light, control animals spent less time on white horizontal surfaces, spent proportionally more time active and traveled greater distances than they did under red light ($P < 0.0001$).

Novelty of association may be 1 explanation for the failure of *M. moniliformis* to alter *D. punctata* behavior under experimental conditions. In other words, assuming that parasite-induced behavioral alterations are species-specific adaptations for parasite transmission, it could be argued that the *D. punctata–M. moniliformis* association is peculiar to our laboratory and has not experienced the selective regime necessary to generate such alterations. There are problems with such a broad assumption that are beyond the scope of this report (see Moore and Gotelli, 1990). In addition, we doubt the "novelty" explanation to be relevant for the following reasons: not only does *M. moniliformis* develop successfully in *D. punctata,* but *D. punctata* probably encounters *M. moniliformis* in nature, although the extent to which *D. punctata* participates in the natural life cycle is unknown. Naturally infected *D. punctata* have not been reported; to our knowledge, they have not been sought. The cockroach can live near human domiciles, and both rats (Ash, 1962) and *Periplaneta americana* (Schaefer, 1970) on the Hawaiian islands are known to harbor *M. moniliformis.* Thus, all elements for *M. moniliformis* life cycle completion are present in the area, and *D. punctata* is likely to be exposed to shelled acanthors and to rat predation.

The failure of *M. moniliformis* to alter *D. punctata* behavior probably is not the result of inappropriate behavioral tests. Infection with *M. moniliformis* has resulted in behavioral alteration in every other arthropod examined to date (Moore, 1984), including 4 other cockroach species (Carmichael and Moore, 1991; Gotelli and Moore, 1992; Moore and Gotelli, 1992). The responses we investigated are commonly altered and can be important components of host survival.

Additional field studies of *D. punctata* are needed for understanding the possible relevance of ecological traits to the apparent absence of parasite-induced behavioral alterations. The absence of altered host behavior is in itself signif-

icant; it calls into question the generalization that acanthocephalans always are associated with behavioral alterations (Moore, 1984). Although *M. moniliformis* alters cockroach behavior, this is neither uniform nor inevitable across host species. Additional comparative study may illuminate evolutionary aspects of behavioral alteration; the absence of alteration in some species could well be critical to this understanding.

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Changes in Fibroblast-derived Trypomastigotes of *Trypanosoma cruzi* during Long-term Culture

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ABSTRACT: Fibroblast-derived trypomastigotes (FDTs) of *Trypanosoma cruzi* that had been in culture for extended periods of time were found to differ in their ability to proliferate in culture when compared to blood-form trypomastigotes (BFTs) and FDTs that had been recently established from blood-forms. "Old" FDTs transform into amastigotes/spheromastigotes and epimastigotes and readily incorporate [^{3}H]thymidine in medium alone or in the presence of mouse spleen cells, whereas "new" FDTs and BFTs did not incorporate [^{3}H]thymidine although they did transform in culture. These differences should be considered when FDTs are used for physiologic and immunologic studies of *T. cruzi.*

Currently there are several methods available that provide means to obtain predominantly amastigotes, epimastigotes, or trypomastigotes of *Trypanosoma cruzi* (Pan, 1978; Hudson et al., 1984; Andrews et al., 1987; Rondinelli et al., 1988). For those examining the immunology of natural or experimental infections, it is of particular significance to examine responses to living trypomastigotes or specific antigens of trypomastigotes. These trypomastigotes generally are obtained from blood of infected mice (Budzko and Kierszenbaum, 1974) or as fibroblast-derived trypomastigotes (FDTs) from cultures (Kuhn and Murnane, 1977).

In the present study, we report that FDTs that have been grown in PSC3H murine fibroblasts (Gooding, 1977) for an extended period readily