

Behavior, Color Changes, and Predation Risk Induced by Acanthocephalan Parasitism in the Caribbean Termite *Nasutitermes acajutlae*

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ABSTRACT.—We documented color and behavior changes of workers of the Caribbean termite *Nasutitermes acajutlae* infected with acanthocephalan parasites, and measured differential predation by *Anolis* lizards on parasitized workers. We broke foraging trails and examined two measures of behavioral change associated with parasitism: the time lapse until first appearance at the broken trail and the time focal animals spent exposed. We compared these variables among parasitized workers, unparasitized workers from parasitized colonies, and workers from unparasitized colonies. We determined if color differed among parasitized workers, unparasitized workers, and unparasitized soldiers by comparing head and body color against a color chart. Finally, we examined if lizard predators preferred parasitized workers over unparasitized workers in choice tests. Parasitized workers arrived at trail breaks significantly earlier and spent significantly more time exposed than unparasitized workers. In choice trials, lizards were significantly more likely to choose parasitized workers. We conclude that changes associated with parasitism increase the likelihood that termites will be eaten by the definitive host of the parasite. To our knowledge, acanthocephalans have not previously been reported to alter the appearance of insects or other terrestrial arthropods, nor have any parasites been reported to alter behavior or appearance of termites.

INTRODUCTION

Parasitism often decreases the survival of the host organism. Parasites may affect survival if parasitized individuals succumb to adverse environmental conditions, are excluded from resources, are directly killed by competitors, or are more susceptible to predation. Because it is often difficult to determine the cause of decreased survival, most researchers have used disappearance from a population as an indicator of mortality (Grenfell and Gulland, 1995). Few studies have documented the mechanism through which parasitism increases mortality under natural conditions, and most of these studies have focussed on predation risk.

One of us (PR) discovered an acanthocephalan (Family Oligacanthorhynchidae) while examining lipids in the termite *Nasutitermes acajutlae*. Acanthocephalans are obligate parasites, often associated with behavior modification leading to increased predation of their hosts. *Nasutitermes* is the

largest genus of termites and is ubiquitous throughout the tropics. *Nasutitermes acajutlae* is widespread through much of the eastern Caribbean and comprises most of the termite biomass on our study sites (pers. obs., Jones and Nalepa, 2002; Thorne et al., 1994).

The genus *Nasutitermes* has three morphologically-distinct castes: reproductives, workers, and soldiers; each with a number of easily identifiable stages. Male and female reproductives leave their colony at the beginning of their adult lives, during a nuptial flight, and after establishing a new colony produce workers, soldiers, and another generation of winged reproductives. Workers forage and feed other castes and immature workers; they also build and maintain the nest and the enclosed foraging trails. Most workers are non-reproductive females and remain workers throughout their lives. Non-reproductive males have worker morphology for 1-2 instars and during this time perform many of the same

tasks as workers. Male workers occur much less frequently and are much smaller than female workers; they usually become soldiers by the second adult molt (Lenz and Wescott, 1985). Soldiers defend the nest against competitors and predators; they have a long, pointed proboscis (nasute) that can squirt a sticky defense compound. Soldiers have reduced jaws, cannot ingest wood directly, and rely on workers to feed them. Workers are typically surrounded by soldiers while foraging (Traniello and Busher 1985).

Adult acanthocephalans live in the intestine of the definitive host and shed eggs with the hosts' feces. Intermediate hosts become infected by eating infective eggs. Transmission from the intermediate host to the definitive host occurs via predation and may involve a paratenic or transport host (a host that eats the first intermediate host and is eaten later by the definitive host). The parasite does not develop further in the transport host. Larval and adult stages of acanthocephalans are host specific; larvae may also encyst in unsuitable (dead end) hosts.

Although workers and soldiers of *N. acajutlae* can become infected by acanthocephalans, over 99 % of infected termites are female workers belonging to instars 3-5 (pers. obs., based on dissections). Infection most likely occurs through ingestion of feces from the definitive host, and soldiers presumably become infected while having small worker morphology. Because they are most common, we only used female workers in this study. Our field observations suggested that parasitized workers left the safety of the enclosed nest and foraging trails more frequently and seemed lighter in color than unparasitized workers (Fig. 1; we can visually distinguish the two groups with over 95 % accuracy). We have also observed that crested anoles (*Anolis cristatellus*) and barred anoles (*A. stratulus*) eat isolated workers, rarely eat isolated soldiers, and take longer to select workers when workers are mixed with soldiers. This suggests that lizards avoid soldiers and that they cannot easily distinguish between workers and soldiers. Because workers are usually surrounded by soldiers

when they are outside of the nest, and they resemble soldiers in terms of coloration, it seems likely that a light-colored worker would be easier for a visual predator to distinguish from soldiers. We tested three predictions of the hypothesis that acanthocephalan parasitism increases predation risk: 1) parasitized workers behave in a way that increases their exposure to predation; 2) there is a color difference between parasitized workers and unparasitized workers; 3) parasitized workers are more susceptible to predation by visual predators than unparasitized workers.

MATERIALS AND METHODS

Study area

The study took place on St. Thomas (19 N, 25 W) and St. John (18 N, 24 W), U.S. Virgin Islands. These islands are small and steep (82.9 km², 460 m elev. and 49.2 km², 370 m elev., respectively). They are dry islands, with rainfall below 130 cm per year, and temperatures range between 21-35 °C. *Nasutitermes acajutlae* colonies occur throughout both islands but their density varies with habitat.

Exposure of parasitized termites during trail breaks

This portion of the study was conducted during August 2001. Data were collected from two parasitized and two unparasitized colonies on St. Thomas and four parasitized and four unparasitized colonies on St. John. Because lizards and termites are less active during the hottest part of the day, we conducted all behavioral trials between 16:00 hrs and sunset. A small section (Mean = 28 cm²; Range = 18-42 cm²) of a foraging trail was scraped away completely and all termites were removed from the exposed area. We examined two measures of predation risk: time until appearance of the first parasitized and the first unparasitized worker, and duration of exposure. We observed the first two parasitized and two unparasitized workers that emerged from the breaks. Each worker was observed from the time its entire body was exposed until it



FIG. 1. Top photo: parasitized nest with exposed parasitized workers (white dots) and unparasitized termites (brown dots). Bottom photo: parasitized workers (light-colored), unparasitized workers (dark-colored), and soldiers (dark-colored, pointed heads).

reentered the trail and disappeared. The average time spent exposed by the two focal animals of each category (parasitized and unparasitized) was used for analysis. Both measures of predation risk were compared among parasitized termites, unparasitized termites from parasitized colonies, and unparasitized termites from unparasitized colonies.

Color differences among parasitized workers, unparasitized workers and soldiers

In October 1996 we collected data on body color from one St. Thomas colony. All termites used in assays of color change and predation risk (see below) were collected between 0900 and 1100 hr, either from foraging trails adjacent to nests or from nests. Termites were transported to the laboratory in covered plastic containers and then sorted into Petri dishes by caste (soldiers or workers). All termites were used on the day they were collected. None of the soldiers used during the trials were parasitized.

To document color differences we collected 10 individuals of each of three termite groups (parasitized workers, unparasitized workers, and unparasitized soldiers). To obtain an unbiased sample, we collected the first 10 lighter and the first 10 darker large workers we encountered. No attempt was made to select the lightest and darkest workers. Because the head and body of *N. acajutlae* has different shades of brown, we determined head and body color separately. We decapitated each termite and placed the head and body individually into numbered glass test-tubes. We haphazardly placed the heads and bodies into the tubes in order to randomize the order in which observers encountered termite types. Termite color did not change noticeably after they were killed. Observers compared termites to a color chart consisting of 15 shades of earth tones ranging from white (number 1) to dark brown (number 15). All termite bodies were examined for acanthocephalans at the end of trials. Three unexperienced observers compared the content of all 60 test-tubes to the color chart. We used three observers to minimize observer error; the three separate

values for each termite head or body were averaged and the averages were compared using ANOVA. If ANOVA models were significant, *t*-tests were used for pairwise comparisons and *p*-values were corrected using the Bonferroni method (Zar, 1999).

Predation risk to exposed termites

Studies of lizard predation were conducted on St. Thomas from February to April, 1996. To test if parasitism increased the risk of workers to predation by a visual predator, we offered anoles a Petri dish containing soldiers and workers. So that none of the termites would be camouflaged, we lined the Petri dishes with a shade of light brown paper intermediate in color between the dark unparasitized and the light parasitized termites. The background we used for the test was also within the range of natural backgrounds. We placed 25-30 soldiers, two parasitized, and two unparasitized workers into each dish. We used termites from eight nests in this test; all termites in any given dish came from the same nest. We placed the dishes in the field on or near logs or rocks, in places where we had observed lizards. Observers stood approx. 3 m from Petri dishes. We waited until a lizard came to the dish (10-15 min) and took one bite. If a soldier was eaten first, we allowed the lizard to take bites until it had eaten one worker. We recorded whether the worker eaten first was parasitized or unparasitized. After one worker had been eaten, we discarded all remaining workers and any injured soldiers. We replaced the workers with new individuals from the same nest and moved the dish to a new location. We placed the dishes at least 4 m from the previous location, never used a location more than once, and carefully observed lizards to ensure that no lizard came to a dish more than once. Because there were equal numbers of parasitized and unparasitized workers in each dish, we assumed that lizards had an equal probability of selecting a parasitized or unparasitized worker.

To test the assumption that lizards avoid eating soldiers, we compared the number of times a lizard ate a soldier before eating

a worker with the number expected if the lizard selected soldiers by chance alone.

RESULTS

Exposure during trail breaks

We observed six parasitized and six unparasitized colonies but due to observer error data about time until first appearance was collected for five unparasitized colonies. Parasitized workers appeared in trail breaks significantly sooner than unparasitized nestmates or workers in unparasitized colonies (Kruskal Wallace = 8.868, $p = 0.012$, $df = 2$; Fig. 2). Parasitized focal animals spent significantly more time in exposed areas than unparasitized nestmates or workers in unparasitized colonies (Kruskal Wallace = 6.924, $p = 0.031$, $df = 2$; Fig. 3).

Color differences

The range of head colors was 5.7-8.7 for parasitized workers, 7.7-14.3 for unparasitized workers, and 12.7-15 for soldiers. The range of body colors was 6.3-11 for parasitized workers, 8.7-14.3 for unparasitized workers, and 10-12.7 for soldiers. There were significant differences among the three groups in head color ($F = 114.3$, $p < 0.001$, $df = 1,28$, Fig. 4A) and body color

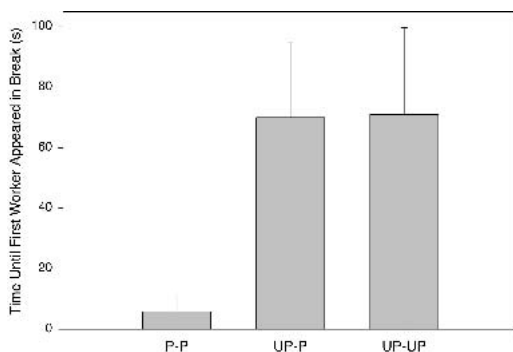


FIG. 2. Time (s) until the first parasitized and unparasitized worker appeared in broken active trails (Mean + SE). P-P: parasitized workers from parasitized colonies; UP-P: unparasitized workers from parasitized colonies; UP-UP: unparasitized workers from unparasitized colonies. $N = 6$ for P-P and UP-P; $N = 5$ for UP-UP. Kruskal Wallace = 8.868, $P = 0.012$, $df = 2$.

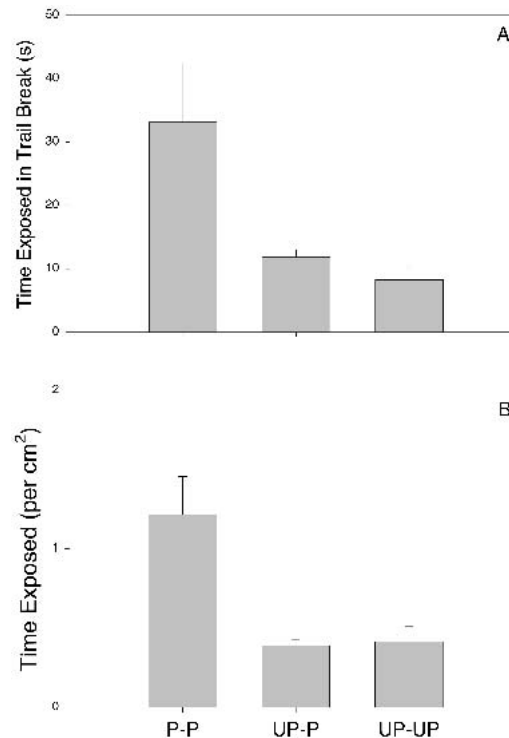


FIG. 3. Time workers spent exposed in trail breaks (Mean + SE). Total uncorrected time (A). Time spent exposed in trail breaks divided by the size of the break (B). P-P: parasitized workers from parasitized colonies; UP-P: unparasitized workers from parasitized colonies; UP-UP: unparasitized workers from unparasitized colonies. $N = 6$ in all categories. Kruskal Wallace = 6.924, $P = 0.031$, $df = 2$; based on data in (B).

($F = 8.81$, $p < 0.01$, $df = 1,28$, Fig. 4B). Parasitized workers were significantly lighter than unparasitized workers ($t=6.41$, $p < 0.005$, head; $t=4.28$, $p < 0.005$, body). The heads of unparasitized workers were significantly lighter in color than those of soldiers ($t=3.47$, $P < 0.006$), but there were no significant differences between body color in these two groups ($t=1.65$, $p > 0.20$).

Predation risk and avoidance of soldiers

Parasitized workers were eaten before unparasitized workers in 16 of 20 trials ($p < 0.005$; Fisher's exact test). All lizards that came to dishes belonged to *A. cristatellus* ($N=17$) or *A. stratulus* ($N=3$). Eighty-six to 88% (25/29 – 30/34) of the termites in each Petri dish were soldiers. By chance alone,

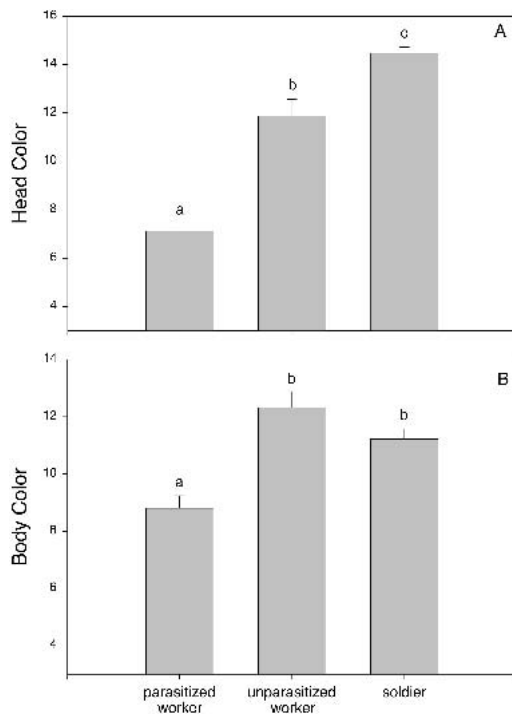


FIG. 4. Head (A) and body (B) color of parasitized workers, unparasitized workers and unparasitized soldiers. Colors were assessed by three inexperienced observers that compared heads and bodies to color charts. Data from the three observers were averaged. Each bar represents the mean (+SE) of 10 termites, letters above bars show groups that differ significantly from each other (*t*-test). Heads and bodies were not compared to each other, only to other heads or bodies.

soldiers should have been eaten first in 17.2 trials (86 % of 20), but they were eaten before workers in only 2 of 20 trials ($p < 0.001$; Fisher's exact test). Lizards that ate soldiers first were juveniles. We believe that the visual cues presented in our Petri dishes and those presented under natural conditions are similar. For example, within 5-10 min following a human-induced trail-break, soldiers and workers move along the uncovered trail as if it were still covered. When lizards arrive (usually within 5 min), they stand adjacent to the uncovered trail, looking down on the termites. The termites in the Petri dishes walked in circles around the perimeter of the dish. As at trail breaks, lizards approached the dish, stood adjacent to it and looked down on prey walking past them.

DISCUSSION

Parasitized workers behaved in a way that increases exposure to predation: they appeared in the breaks sooner and they spent more time exposed. There were no differences between the behavior of unparasitized workers from parasitized and those from unparasitized colonies, confirming that behaviors are associated with parasitism and not with nests. The proximate mechanism for these behavioral changes is unclear. Parasitized termites may prefer warmer (e.g., behavioral fever, Lefcort and Eiger, 1993) or drier environments that occur outside the nest. Changes in microhabitat preference have been documented in the intermediate host of several acanthocephalans (reviewed by Moore, 2001). Alternatively, the response of parasitized workers to alarm pheromones may be more similar to that of soldiers than to that of unparasitized workers. Soldiers generally respond first to disturbances; older workers arrive later (Roisin et al., 1990).

Heads and bodies of parasitized workers from our test colony were significantly lighter in color. We have dissected parasitized workers from 12 additional colonies on St. Thomas and 26 additional colonies on St. John and have found the same color change in each colony. Field trials demonstrated that anoles select parasitized workers over unparasitized workers when workers were in the presence of soldiers. Lizards ate fewer soldiers than would be expected by chance, supporting the hypothesis that soldiers are avoided. Although lizards in our choice tests may have used a cue other than color to distinguish parasitized from unparasitized termites, neither group of termites exhibited uncharacteristic behaviors and all walked around the perimeter of the dish at the same pace. Lizards may be able to distinguish parasitized and unparasitized termites based on olfactory cues but it seems unlikely that a lizard could pick one parasitized worker out of the mix of cues associated with 30 termites in close quarters, especially when all termites were in constant motion.

Color changes occur in the intermediate hosts of other acanthocephalans, including

aquatic amphipods (Hindsbo, 1972; Bakker, et al. 1997) and aquatic isopods (Camp and Huizinga, 1979; Oettinger and Nickol, 1981; 1982). Cestodes (Plateaux, 1972) and gregarines (Crosland, 1988) have been reported to alter color in ants. To our knowledge, acanthocephalans have not previously been reported to alter the appearance of insects or other terrestrial arthropods, nor have any parasites been reported to alter behavior or appearance of termites.

While parasitism clearly increases predation risk when trails are broken, it is unclear how frequently this occurs. Although we rarely saw open trails in undisturbed nests, we frequently saw evidence of recent repairs (patches of newly laid trail are darker than older trail), suggesting that our human-induced breaks mimic natural conditions. Trails are brittle and frequently stretch across open spaces between two branches; presumably they are susceptible to breakage by animals, falling branches, or wind. There are no animals in the Virgin Islands that open termite nests as a regular part of their diet, but we have observed pearly-eyed thrashers (*Margarops fuscatus*) attacking colonies.

Although it is unclear how often workers are eaten when trails break, we have evidence that several species in three vertebrate classes eat parasitized termites. In 1995, we fed parasitized workers to a captive adult female *A. cristatellus* to determine if this species was the definitive host of the acanthocephalan. The parasites did not complete development in the intestine but appeared instead as conspicuous nodules under the skin of the lizard. Dissection of this and two naturally-infected lizards confirmed that the nodules were encysted larvae of the acanthocephalan (B. B. Nickol, pers. comm.). We examined lizards opportunistically at parasitized and unparasitized colonies and found that 6 of 9 *A. cristatellus* at parasitized colonies, and 2 of 18 *A. cristatellus* at unparasitized colonies, had nodules. We saw similar nodules in 2 *A. stratulus* near parasitized nests and found encysted larvae in the body cavities of *M. fuscatus* and the Indian mongoose (*Herpestes auro-punctatus*).

We have not found the definitive host of

the termite acanthocephalan, but based on parasite taxonomy it is apparently a bird (Brent B. Nickol, pers. comm.). Although we do not know if changes in behavior and color increase transmission to the definitive host, parasites clearly increase the visibility and exposure of infected workers.

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LITERATURE CITED

- Bakker, T. C. M., D. Mazzi and S. Zala. 1997. Parasite-induced changes in behavior and color make *Gammarus pulex* more prone to fish predation. *Ecology* 78:1098-1104.
- Camp, J. W. and H. W. Huizinga. 1979. Altered color, behavior and predation susceptibility of the isopod *Asellus intermedius* infected with *Acanthocephalus dirus*. *J. Parasitol.* 65:667-669.
- Crosland, M. W. J. 1988. Effect of a gregarine parasite on the color of *Myrmecia pilosula* (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.* 81:481-484.
- Grenfell, B. T. and F. M. D. Gulland. 1995. Introduction: ecological impact of parasitism on wildlife host populations. *Parasitology* 111:S3-S14.
- Hindsbo, O. 1972. Effects of *Polymorphus* (Acanthocephala) on colour and behaviour of *Gammarus lacustris*. *Nature* 238:333.
- Jones, S. C. and C. A. Nalepa. 2002. Survey of the termites (Isoptera: Kalotermitidae, Rhinotermitidae, Termitidae) of St. John, U.S. Virgin Islands. *Sociobiology* 39:155-163.
- Lefcort, H. and S. M. Eiger. 1993. Antipredatory behaviour of feverish tadpoles: implications for pathogen transmission. *Behaviour* 126:13-27.
- Lenz, M. and M. Wescott. 1985. Homeostatic mechanisms affecting caste composition in groups of *Nasutitermes nigriceps* (Isoptera: Termitidae) exposed

- to a juvenile hormone analogue. *In*: Caste Differentiation in Social Insects, J. A. L. Watson et al. (Eds.), pp. 251-266. Pergamon Press, Elmsford, NY.
- Moore, J. 2001. Parasites and the Behavior of Animals. Oxford Series in Ecology and Evolution. Oxford University Press, Oxford. 295 pp.
- Oetinger, D. F. and B. B. Nickol. 1981. Effects of acanthocephalans on pigmentation of freshwater isopods. *J. Parasitol.* 67:672-684.
- Oetinger, D. F. and B. B. Nickol. 1982. Spectrophotometric characterization of integumental pigments from uninfected and *Acanthocephalus dirus*-infected *Asellus intermedius*. *J. Parasitol.* 68:270-275.
- Plateaux, P. L. 1972. Sur les modifications produites chez une fourmi par la présence d'un parasite cestode. *Ann. Sciences Naturelles Zoologie, Paris* 14:203-220.
- Roisin, L. S., Everaerts, C., J. M. Pasteels and O. Bonnard. 1990. Caste-dependent reactions to soldier defensive secretions and chiral alarm/recruitment pheromone in *Nasutitermes princeps*. *J. Chem. Ecol.* 16:2865-2875.
- Thorne, B. L., Haverty, M. I., and Collins, M. 1994. Taxonomy and biogeography of *Nasutitermes acajutlae* and *N. nigriceps* (Isoptera: Termitidae) in the Caribbean and central America. *Ann. Entomol. Soc. Amer.* 87:762-770.
- Traniello, J. F. A. and C. Busher. 1985. Chemical regulation of polyethism during foraging in the neotropical termite *Nasutitermes costalis*. *J. Chem. Ecol.* 11:319-332.
- Zar, J. H. Biostatistical Analysis, 4th Edition. Prentice Hall. Upper Saddle River, USA, 663 pp.