J. Parasitol., 98(1), 2012, pp. 226–228

Exoskeletal Thinning in *Cephalotes atratus* Ants (Hymenoptera: Formicidae) Parasitized by *Myrmeconema neotropicum* (Nematoda: Tetradonematidae)

Robin M. Verble, Ashley D. Meyer, Maurice G. Kleve, and Stephen P. Yanoviak*, Department of Biology, University of Arkansas at Little Rock, 2801 South University Avenue, Little Rock, Arkansas 72204; *To whom correspondence should be addressed. *e-mail: spyanoviak@ualr.edu*

ABSTRACT: Some parasites modify the color of their arthropod hosts, presumably to facilitate transmission to a new host. Mechanisms for such changes often are unknown, but altered exoskeletal color in adult insects typically occurs via structural modifications or redistribution of pigments. Here, we examine the cuticle structure of workers of the Neotropical canopy ant *Cephalotes atratus* infected with the nematode *Myrmeconema neotropicum*. We hypothesized that the conspicuous red color of the gaster (the globular posterior body region) of infected ants results from structural changes, specifically localized exoskeletal thinning. We used scanning electron microscopy to quantify the thickness of gaster cuticle in healthy and infected ants. For comparison, we also measured the cuticle thickness of the head of each ant, which is black in both infected and healthy individuals. The gaster cuticle was 23% thinner in infected ants (average ±SE: 14.8 ± 1.02 μm) versus healthy ants (19.2 ± 0.65 μm) after correcting for body size. In contrast, the thickness of the head exoskeleton was similar among groups. We conclude that parasite-induced thinning of the exoskeleton is associated with the red color of the gaster. Other mechanisms, including translocation or leaching of melanin (by the ant or the parasite, respectively) may operate in concert with thinning to effect the color change, and would be an appropriate extension of this research.

Parasites often influence host phenotypes in ways that appear to facilitate their transmission to final hosts (e.g., Schmid-Hempel, 1998; Moore, 2002). Whereas parasite effects on host physiology and ecology may be subtle (Shik et al., 2011), parasite-induced changes in the behavior and appearance of arthropods often are dramatic. Some of the more striking examples of such effects specifically involve changes in the color of the integument. Conspicuous exoskeletal color changes are found in isopods infected with acanthocephalans (e.g., Oetinger and Nickol, 1981, 1982), and ants infected with cestodes (Muir, 1954; Trabalon et al., 2000), flukes (Carney, 1969), nematodes (Lee, 1957; Yanoviak et al., 2008), and gregarines (Crosland, 1988). The mechanisms for such changes are not well resolved in most cases (but see Oetinger and Nickol, 1981, 1982).

Exoskeletal colors in insects exist by deposition of pigment compounds during development, e.g., melanin and ommochromes, or through interactions between incident light and cuticle morphology, i.e., structural color (Klowden, 2007). Changes in coloration are uncommon in adult insects and generally require significant reorganization of exoskeletal structure or redistribution of pigments (Fuzeau-Braesch, 1985; Chapman, 1998). Thus, parasite-induced color changes in arthropods are not trivial phenomena. They generally occur via 3 mechanisms that are not mutually exclusive. First, morphological changes include thinning of the exoskeleton or exposure of pleural membranes by distension of the body wall, as in fluke–infected *Camponotus* spp. ants (Carney, 1969). Second, biochemical changes result in pigment redistribution or removal (Muir, 1954; Klowden, 2007). Finally, the change in appearance may occur by indirect mechanisms, resulting entirely from the color of the parasite itself (Sanchez-Peña et al., 1993) or via symbiosis between a parasite and a third organism (Fenton et al., 2011).

Here, we explore a potential mechanism for the dramatic change in color that occurs in workers of the Neotropical canopy ant *Cephalotes atratus*, infected with the nematode *Myrmeconema neotropicum*. Details of this host–parasite association are provided elsewhere (Poinar and Yanoviak, 2008; Yanoviak et al., 2008; Shik et al., 2011). Briefly, a *C. atratus* larva becomes infected when it is exposed to bird feces contaminated with nematode eggs. As the ant matures, the nematodes migrate to its gaster (the globular posterior body region), which ultimately becomes a conspicuous red vessel containing hundreds of nematode eggs in the adult ant. Healthy ants are completely black, as are infected ants upon emergence from the pupal stage. Gradual reddening of the gaster coincides with development of the nematode embryos and temporal polyethism in the adult ant (see Corn, 1980), such that maximum redness occurs when the nematodes are at peak infectivity and the ant is spending most of its time foraging. Consequently, it is likely that birds act as paratenic hosts (Moore, 2002), completing the cycle by mistakenly consuming the berry-like red gasters and transmitting the nematodes to new ant colonies in their feces (Yanoviak et al., 2008).

Based on our knowledge of the *C. atratus–M. neotropicum* host–parasite system, we pose 3 mechanistic hypotheses for the color change in infected ants. First, the increased gaster redness may be due to parasite-induced deposition of red pigments in the cuticle, e.g., carotenoids and ommochromes (Chapman, 1998). We know of no examples of such an effect caused by parasites. Second, given that parasites often influence exoskeletal structure in their arthropod hosts (Hepburn, 1985), and that insect cuticle includes proteins, lipids, and other compounds of potential nutritional value (Filshie, 1982), *M. neotropicum* may cause thinning of the ant exoskeleton by extracting such components during development (cf. Oetinger and Nickol, 1982). Significant thinning would allow light interference with cuticle layers, resulting in a translucent amber appearance. This effect occurs naturally where patches of thin cuticle exist on healthy *C. atratus* workers, e.g., the frontal lobes above the eyes. Finally, the black color of healthy *C. atratus* workers presumably is due to an abundance of cuticular melanin. Although melanin and related compounds are cross-linked in the cuticle during sclerotization (Andersen, 1985), melanin is both nutritionally valuable and used by arthropods to encapsulate pathogens (Klowden, 2007). Thus, the red gasters of infected ants may be due to selective physiological removal of melanin from the exoskeleton, perhaps via active relocation by the ant, or by extraction and subsequent consumption by the developing parasite embryos, or both. Here, we focus on the second hypothesis. Specifically, we predict that the gaster exoskeleton is significantly thinner in infected ants. We further expect that the thickness of normal, black exoskeleton of infected ants will not differ from similar exoskeletons on healthy ants.

Fieldwork for this project was conducted on Barro Colorado Island (BCI), Panama (9.16°N, 79.85°W), as part of prior studies on this system (Poinar and Yanoviak, 2008; Yanoviak et al., 2008) and during more recent visits (May 2009, 2010). The BCI forest is classified as seasonally moist, receiving ca. 2,700 mm of rain per year, punctuated by a distinct 3-mo dry season. Leigh et al. (1996) provide additional details regarding the climate and biology of the site.

Infected and uninfected *C. atratus* workers were collected from 2 colonies separated by >100 m. In each case, the home tree of the colony was climbed with the use of the single rope technique (Perry, 1978) and baits consisting of canned tuna (in water) mixed with honey were placed on branches and lianas. Ants were allowed to accumulate at baits for about 1 hr, then infected workers (indicated by their red gasters) and a representative sample of healthy workers (entirely black) were collected and placed into 95% ethanol. Voucher specimens were deposited in the synoptic insect collection at the Smithsonian Tropical Research Institute (Balboa, Panama), the Fairchild Museum (University of Panama, Panama City, Panama), and the Watson Entomological Museum (University of Arkansas at Little Rock, Little Rock, Arkansas).

We measured head width (HW, maximum width posterior to eyes; spines and eyes excluded), head length (HL, excluding mandibles), thorax length (TL, distance from the anterior margin of the pronotum to the posterior margin of the propodeum), pronotum width (PW, measured immediately posterior to the pronotal spines), and foretibia length (FL,

DOI: 10.1645/GE-2847.1

TABLE I. Averages (±SE) of morphological variables measured on healthy and infected *Cephalotes atratus* workers. HW = head width (mm), HL = head length (mm), PW = pronotum width (mm), T1L = foretibia length (mm), TxL = thorax length (mm). See text for measurement details. Degrees of freedom (df) vary because of missing data. Mass (mg) measurements exclude gasters.

	Healthy	Infected	df	*t*	*P*
HW	2.94 ± 0.103	2.64 ± 0.109	34	1.98	0.055
HL	2.50 ± 0.091	2.28 ± 0.097	34	1.66	0.112
PW	1.87 ± 0.078	1.68 ± 0.084	28	1.69	0.102
T1L	1.79 ± 0.054	1.69 ± 0.058	34	1.24	0.222
TxL	3.89 ± 0.101	3.69 ± 0.112	29	1.39	0.176
Mass	10.6 ± 0.65	7.7 ± 0.68	34	3.06	0.004

the distance from the distal margin of the femur to the basal margin of the first tarsomere) on each ant. The dry mass of each ant (gaster excluded) was determined to the nearest 0.1 mg after 24 hr at 75 C.

We used environmental scanning electron microscopy (eSEM; model PSEM II, ASPEX LLC, Delmont, Pennsylvania) to measure exoskeletal thickness. The gaster of each ant was sectioned transversely at its midpoint with a fresh blade. The head of each ant was similarly bisected along its longitudinal axis. The resulting sections were mounted on stubs and positioned such that the thickness of the cut edge could be accurately measured. Specimens were observed at 200–500x magnification. Integument thickness was measured with the use of resident eSEM software at 5 haphazardly selected points (separated by at least 20 μm) along the exposed edge of the gaster dorsum, i.e., abdominal tergum 3, and similarly at 5 points on the longitudinal axis of the exoskeleton of the head, i.e., the frons. The average of the 5 measurements for each body region was used as the datum for each ant (n = 36 gasters, n = 34 heads; heads of 2 infected ants were excluded due to damage during sectioning).

We compared the thickness of the exoskeleton between infected and healthy ants with ANCOVAs, with HW as a covariate. Gaster and head exoskeletal thickness were analyzed separately. We used HW as the covariate rather than mass for 3 reasons: (1) mass measurement excluded the gaster, which consistently weighs more when parasites are present (Yanoviak et al., 2008; Shik et al., 2011); (2) HW was more tightly correlated with other morphometrics than mass (see below); and (3) the thickness of both head and gaster exoskeleton increased with HW, but not with mass.

Analyses were conducted with SAS software (SAS Institute, 2002). All data were tested for homogeneity of variance and normality (Shapiro-Wilk *W* or Kolmogorov *D*) before analysis. Data were log-transformed when necessary to correct variance heterogeneity or improve normality (Sokal and Rohlf, 1995).

Infected ants and healthy ants used in the study were similar in most morphometrics, but infected ants weighed significantly less than healthy ants (gasters excluded; Table I). All morphological variables measured on the ants were significantly correlated with each other and with mass (Table II). As in other studies with *C. atratus* (Corn, 1980; Yanoviak et al., 2008), HW showed the strongest relationship with mass (Table II). However, we observed that HW relates to various other morphometrics better than does mass. Specifically, the average (±SD) correlation coefficient between HW and other metrics excluding mass was significantly higher (0.811 ± 0.1053) than between mass and other metrics, excluding HW (0.766 ± 0.1230; paired $t = 4.03$, df = 7, $P = 0.005$).

TABLE II. Correlation matrix for morphological variables measured on healthy (boldface values) and infected *Cephalotes atratus* workers. Within treatments, all pairwise comparisons are significant ($P < 0.032$). HW = head width (mm), HL = head length (mm), PW = pronotum width (mm), T1L = foretibia length (mm), TxL = thorax length (mm). Mass (mg) measurements exclude gasters.

	HW	HL	PW	T1L	TxL	Mass
HW	–	0.836	0.914	0.812	0.833	0.950
HL	**0.896**	–	0.927	0.619	0.931	0.781
PW	**0.897**	**0.914**	–	0.686	0.954	0.865
T1L	**0.657**	**0.660**	**0.740**	–	0.601	0.723
TxL	**0.645**	**0.756**	**0.702**	**0.572**	–	0.796
Mass	**0.961**	**0.864**	**0.914**	**0.615**	**0.568**	–

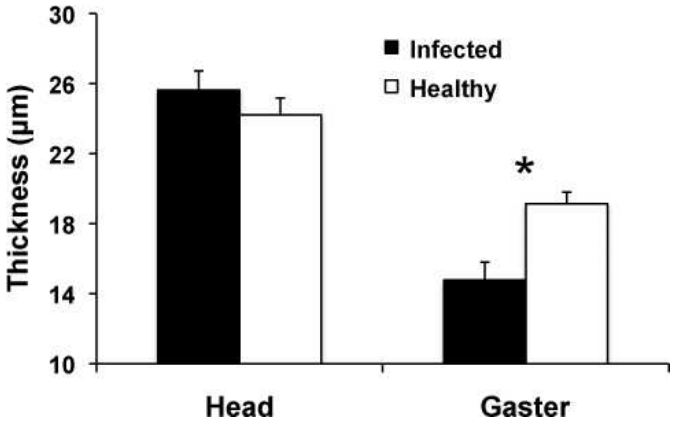

FIGURE 1. Average (+SE) exoskeletal thickness of the head and gaster of infected and healthy *Cephalotes atratus*. Head n = 34, gaster n = 36, * = $P < 0.05$.

The thickness of the head exoskeleton of infected ants did not differ from that of healthy ants when corrected for body size (Fig. 1; $F_{1,30} = 0.95$, $P = 0.34$). However, the gaster exoskeleton of infected ants was significantly thinner and marginally less variable than that of healthy ants (Fig. 1; $t = 3.52$, df = 34, $P = 0.0012$; Bartlett's test: $F = 3.87$, $P < 0.05$). Covariance slopes were heterogeneous in the analysis of gaster exoskeleton thickness ($F_{1,30} = 6.87$, $P = 0.013$); thus we compared means with a *t*-test.

Our results support the hypothesis that the red appearance of *C. atratus* gasters containing *M. neotropicum* is associated with thinning of the exoskeleton. This conclusion is reinforced by the lack of difference in thickness of black exoskeleton between healthy and infected ants. Presumably, nematode embryos biochemically erode the host ant cuticle from within as they develop (St. Leger, 1993), facilitating increased light transmission through the gaster exoskeleton, and consequently a red appearance. The relatively low variance in gaster exoskeleton thickness among infected ants suggests that the erosive process is tightly regulated to prevent collapse or premature rupture of the gaster, which would kill the ant and catastrophically interfere with transmission of the parasite to new colonies. We hypothesize that the biochemical process of exoskeletal erosion in this case is inhibited by light. Such a mechanism would facilitate suspension of the erosive process at a point that does not prematurely destroy the gaster. This notion remains to be tested.

All evidence to date provides no support for our first hypothesis—that the red color of the gaster cuticle in infected ants is the result of deposition of pigments by the parasites. Indeed, it is more likely that parasites extract such compounds from their hosts (Oetinger and Nickol, 1982). However, we cannot completely exclude our third hypothesis—the possibility that translocation or leaching of melanin (by the ant or the parasite, respectively) operate in combination with exoskeletal thinning to produce the red color in *C. atratus*. Melanin plays important roles in insect defense against pathogens (Wilson et al., 2001) and in the encapsulation of parasitoids (Klowden, 2007). Our observations suggest that some translocation is indeed occurring; ants in the latter stages of infection show partial reddening of the femurs with no obvious effect on the integrity of the femoral exoskeleton (Yanoviak et al., 2008).

The *C. atratus–M. neotropicum* symbiosis exemplifies the diverse and complicated effects that parasites can have on their hosts, and it is likely that more detailed investigation of the biochemical aspects of this relationship will uncover interesting patterns. Other appropriate extensions of this work could include comparative study of the mechanisms underlying the color change in *Pogonomyrmex* spp. ants that serve as the intermediate host for the toad-infecting nematode *Skrjabinoptera phrynosoma* (Lee, 1957). This host–parasite association is superficially similar to the *C. atratus–M. neotropicum* system in that the ants are infected during the larval stage, and the gasters of parasitized worker ants are lighter in

color (Lee, 1957). As in *C. atratus*, the mechanism of the color change is unknown. Finally, similar reddening of gasters occurs in *Cephalotes christopherseni* infected with nematodes, and occasionally in *C. atratus* infected with insect parasitoids (S. Yanoviak, pers. obs.). The mechanism for the color change in these cases presumably is similar, but has not been investigated.

We thank Oris Acevedo, Belkys Jimenez, Annette Aiello, and the staff of the Smithsonian Tropical Research Institute for facilitating our work on BCI, and for assisting with the acquisition of permits through the Panamanian Autoridad Nacional del Ambiente. Manuela Koinig (UALR) shared her preliminary observations of this system, and Rahul Mehta (University of Central Arkansas) assisted with electron microscopy, imaging, and measurements. This research was supported in part by grant IOS-0843120 to SPY from the National Science Foundation and by an undergraduate research grant to A.D.M. from UALR.

LITERATURE CITED

ANDERSEN, S. O. 1985. Sclerotization and tanning of the cuticle. *In* Comprehensive insect physiology, biochemistry, and pharmacology. Vol. 3, Integument, respiration and circulation, G. A. Kerkut and L. I. Gilbert (eds.). Pergamon Press, Oxford, U.K., p. 59–74.

CARNEY, W. P. 1969. Behavioral and morphological changes in carpenter ants harboring dicrocoeliid metacercariae. The American Midland Naturalist **82:** 605–611.

CHAPMAN, R. F. 1998. The insects: Structure and function. Cambridge University Press, Cambridge, U.K., 788 p.

CORN, M. L. 1980. Polymorphism and polyethism in the neotropical ant, *Cephalotes atratus* (L.). Insectes Sociaux **27:** 29–42.

CROSLAND, M. W. J. 1988. Effect of a gregarine parasite on the color of *Myrmecia pilosula* (Hymenoptera: Formicidae). Annals of the Entomological Society of America **81:** 481–484.

FENTON, A., L. MAGOOLAGAN, Z. KENNEDY, AND K. A. SPENCER. 2011. Parasite-induced warning coloration: A novel form of host manipulation. Animal Behaviour **81:** 417–422.

FILSHIE, B. K. 1982. Fine structure of the cuticle of insects and other arthropods. *In* Insect ultrastructure. Vol. 1. R. C. King and H. Akai (eds.). Plenum Press, New York, New York, p. 281–312.

FUZEAU-BRAESCH, S. 1985. Colour changes. *In* Comprehensive insect physiology, biochemistry, and pharmacology. Vol. 3, Integument, respiration and circulation, G. A. Kerkut and L. I. Gilbert (eds.). Pergamon Press, Oxford, U.K., p. 549–589.

HEPBURN, H. R. 1985. Structure of the integument. *In* Comprehensive insect physiology, biochemistry, and pharmacology. Vol. 3, Integument, respiration and circulation, G. A. Kerkut and L. I. Gilbert (eds.). Pergamon Press, Oxford, U.K., p. 1–58.

KLOWDEN, M. J. 2007. Physiological systems in insects. Academic Press, Burlington, Massachusetts, 415 p.

LEE, S. H. 1957. The life cycle of *Skrjabinoptera phrynosoma* (Ortlepp) Schulz, 1927 (Nematoda: Spiruroidea), a gastric nematode of Texas horned toads, *Phrynosoma cornutum*. Journal of Parasitology **43:** 66–75.

LEIGH, E. G., JR., A. S. RAND, AND D. M. WINDSOR. 1996. The ecology of a tropical forest: Seasonal rhythms and long-term changes, 2nd ed. Smithsonian Institution, Washington, DC, 503 p.

MOORE, J. 2002. Parasites and the behavior of animals. Oxford University Press, New York, New York, 338 p.

MUIR, D. A. 1954. Ants *Myrmica rubra* L. and *M. scabrinodis* Nylander as intermediate hosts of a cestode. Nature **173:** 688–689.

OETINGER, D. F., AND B. B. NICKOL. 1981. Effects of acanthocephalans on pigmentation of freshwater isopods. Journal of Parasitology **67:** 672–684.

———, AND ———. 1982. Developmental relationship between acanthocephalans and altered pigmentation on freshwater isopods. Journal of Parasitology **68:** 463–469.

PERRY, D. R. 1978. A method of access into the crowns of emergent and canopy trees. Biotropica **10:** 155–157

POINAR, G., JR., AND S. P. YANOVIAK. 2008. *Myrmeconema neotropicum* n. g., n. sp., a new tetradonematid nematode parasitizing South American populations of *Cephalotes atratus* (Hymenoptera: Formicidae), with the discovery of an apparent parasite-induced host morph. Systematic Parasitology **69:** 145–153.

SANCHEZ-PEÑA, S. R., A. BAUSCHINGER, AND R. A. HUMBER. 1993. *Myrmicinosporidium durum*, an enigmatic fungal parasite of ants. Journal of Invertebrate Pathology **61:** 90–96.

SAS INSTITUTE, INC. 2002. SAS OnlineDoc 9. SAS Institute, Inc., Cary, North Carolina.

SCHMID-HEMPEL, P. 1998. Parasites in social insects. Princeton University Press, Princeton, New Jersey, 392 p.

SHIK, J. Z., M. KASPARI, AND S. P. YANOVIAK. 2011. Preliminary assessment of metabolic costs of the nematode *Myrmeconema neotropicum* on its host, the tropical ant *Cephalotes atratus*. Journal of Parasitology **97:** 958–959.

SOKAL, R. R., AND F. J. ROHLF. 1995. Biometry. W.H. Freeman and Co., New York, New York, 880 p.

ST. LEGER, R. 1993. Biology and mechanisms of insect-cuticle invasion by deuteromycete fungal pathogens. *In* Parasites and pathogens of insects. Vol. 2, Pathogens, N. E. Beckage, S. N. Thompson, and B. A. Federici (eds.). Academic Press, San Diego, California, p. 211–230.

TRABALON, M., L. PLATEAUX, L. PÉRU, A. G. BAGNÈRES, AND N. HARTMANN. 2000. Modification of morphological characters and cuticular compounds in worker ants *Leptothorax nylanderi* induced by endoparasites *Anomotaenia brevis*. Journal of Insect Physiology **46:** 169–178.

WILSON, K., S. C. COTTER, A. F. REESON, AND J. K. PELL. 2001. Melanism and disease resistance in insects. Ecology Letters **4:** 637–649.

YANOVIAK, S. P., M. KASPARI, R. DUDLEY, AND G. POINAR, JR. 2008. Parasite-induced fruit mimicry in a tropical canopy ant. The American Naturalist **171:** 536–544.

DATE OF PUBLICATION
Volume 98, No. 1, was mailed 29 February 2012