

Natural History Note

Parasite-Induced Fruit Mimicry in a Tropical Canopy Ant

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ABSTRACT: Some parasites modify characteristics of intermediate hosts to facilitate their consumption by subsequent hosts, but examples of parasite-mediated mimicry are rare. Here we report dramatic changes in the appearance and behavior of nematode-parasitized ants such that they resemble ripe fruits in the tropical rain forest canopy. Unlike healthy ants, which are completely black, infected ants have bright red, berry-like gasters full of parasite eggs. The infected gasters are held in a conspicuous elevated position as the ants are walking, and they are easily detached from living ants, which also exhibit reduced defensive responses. This combination of changes presumably makes the infected ants attractive to frugivorous birds, which ingest the red gasters and pass the parasite eggs in their feces. The feces are collected by ants and fed to the developing brood, thus completing the cycle. This is the first documentation of parasites causing apparent fruit mimicry in an animal host to complete their life cycle.

Keywords: behavior, color, frugivory, interaction, nematode, parasitism, *Cephalotes*, *Myrmecconema*.

Parasites can modify characteristics of arthropod hosts in diverse ways to facilitate their transmission to subsequent hosts (Holmes and Bethel 1972; Hurd 1990; Schmid-Hempel 1998; Moore 2002). These modifications often are behavioral, with infected individuals presenting no other obvious phenotypic differences from uninfected conspecifics (Sheiman et al. 2006; reviewed by Moore [2002]). However, frequently the altered behaviors are accompanied by physical changes that make the host more conspicuous, such as an increase in contrast or size. In such cases, the modifications may not be directly involved in facilitation of transmission (Moore 2002) but will be favored by selection when the terminal host is a visual predator prone to consume rare morphs (i.e., oddity selection, novelty selection, or antiapostatic foraging; Curio 1976; Lindström et al. 2001). Many suggestive examples of this increased conspicuousness strategy exist, but most are not supported by field data (Holmes and Bethel 1972; Hurd 1990; Moore 2002).

Whereas increasing the conspicuousness of familiar palatable prey may be a common and effective parasite transmission strategy, there are few cases of parasite-induced mimicry, whereby the appearance of an intermediate host is transformed to the extent that it resembles a completely different organism. The only terrestrial invertebrate example is the trematode *Leucochloridium*, which causes the antennae of infected snails to resemble caterpillars (Moore 2002). When the intermediate host (i.e., the mimic) has a sting or otherwise is distasteful to the subsequent host, such a transformation should be accompanied by a reduction in its defenses. Nematode parasites can have this effect on *Lasius* ants, causing them to lack normal defensive responses and venom production (Gösswald 1930), although mimicry is not involved in this example. To our knowledge, there are no reports of parasites causing arthropods to resemble plant parts or of extreme mimicry combined with a change in host defenses. Here we describe what appears to be such a case in the tropical arboreal ant *Cephalotes atratus* (Formicidae: Myrmicinae).

Parasite-induced physical changes (e.g., distended gaster, intercaste morphology) are fairly common in ants, and

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some parasites modify ant colors as well as anatomy (Schmid-Hempel 1998; Moore 2002). Classic examples include the pale, distended gasters of nematode- and trematode-infected ants (Wheeler 1907; Gösswald 1930; Carney 1969) and the altered color of ants infected with gregarines (Crosland 1988) or cestodes (Heinze et al. 1998; Tralalon et al. 2000). These changes generally involve an overall lightening in color, often resulting from cuticular thinning (Crosland 1988; Tralalon et al. 2000; but see Muir 1954). Ant-associated parasites may cause other generalized morphological effects, such as a reduction or increase in overall body size (Passera 1976; Tralalon et al. 2000).

Physical effects of parasites on ants may act in concert with behavioral changes (e.g., sluggishness, lack of escape response) to facilitate consumption of infected workers by birds or other vertebrates (Carney 1969, 1970; Moore 1995, 2002; Heinze et al. 1998). Generalized alterations of body size are an exception; size polymorphism is common in ants, and the typical range of these changes ($\pm 10\%$ – 20%) is probably irrelevant to enhanced parasite transmission in the absence of altered color and other modifications. The magnitude of physical effects often is independent of parasite loads (Carney 1969; Tralalon et al. 2000), but this may depend on the type of parasite (Crosland 1988) or the type of response measured (e.g., cuticular hydrocarbons; Tralalon et al. 2000). Also, the type or magnitude of phenotypic alterations may differ among closely related host or parasite species (Heinze et al. 1998). The importance of individual or interspecific host variation to parasite transmission is unknown.

In May 2005, we discovered a *C. atratus* colony in Panama that included several foragers with conspicuous red gasters (fig. 1). Like other investigators, we initially assumed that the red coloration represented a new taxon (e.g., a social parasite on *C. atratus*) or a developmental aberration (de Andrade and Baroni Urbani 1999). Dissection of the gaster revealed that it contained hundreds of transparent eggs, each housing a small coiled worm, subsequently determined to represent a new genus of tetradonematid nematode (Poinar and Yanoviak 2008). Nematode infections are not commonly observed in *Cephalotes* (de Andrade and Baroni Urbani 1999), and only one other tetradonematid is known from ants (Nickle and Jouvenaz 1987). Tetradonematids are more typically associated with wood-boring beetles and flies (Zervos 1980; Peloquin and Platzer 1993; Tang et al. 1997). They do not greatly modify the appearance of these insects, and sparse collection records suggest that they are widespread but undersampled (Zervos 1980). Here we describe the dramatic effects of a new tetradonematid parasite on *C. atratus*, which apparently facilitate its transmission to a frugivorous host.

Methods

The majority of the fieldwork was conducted on Barro Colorado Island (BCI), Panama (9.16°N, 79.85°W), during a series of short field trips in May and June of 2005–2007 and November 2005 and 2006 (see Leigh et al. 1996 for a description of the site). Observations from other forests in the Panama Canal Zone (e.g., Gamboa, Pipeline Road, Camino de Cruces) were obtained from a September 2007 survey conducted by S. Price (University of California, Los Angeles). In addition, *Cephalotes atratus* colonies were examined at four lowland locations in the Peruvian Amazon: (1) in and around the city of Iquitos (3.75°S, 73.25°W); (2) along the Iquitos-Nauta highway (4.33°S, 73.53°W; Mäki et al. 2001); (3) at the Amazon Conservatory for Tropical Studies (ACTS) and Explornapo field stations (3.25°S, 72.91°W; Vásquez Martínez 1997; Madigosky and Vatnick 2000); and (4) at the Amazon Conservation Association Los Amigos field station (CICRA) 100 km west of Puerto Maldonado (12.57°S, 70.10°W).

We estimated infection rates in parasitized colonies by counting the number of red-gastered and normal workers visiting baits and returning to the nest via vines (see Poinar and Yanoviak 2008). To ensure the accuracy of this method, 300 completely black foragers were collected from a parasitized colony on BCI and dissected; none was infected with nematodes. Counts used to determine infection rates were conducted on one of the first two days of a field trip, were initiated immediately on arrival at the focal nests, and were separated by >3 weeks. Conspicuous behaviors, in particular gaster flagging, also were noted during these counts (i.e., we recorded the gaster position of ants passing a focal point). The total number of ants observed per colony in a given count ranged from 60 to 620.

In addition to counting foragers, we opened an infected colony occupying a large *Jacaranda copaia* (Bignoniaceae) tree at CICRA in Peru and collected 1,602 third- and fourth-instar larvae, 1,693 worker pupae, 40 male pupae, 40 callow workers, and 131 adult workers. Subsets of the collected larvae and pupae were haphazardly selected for dissection to determine the life cycle of the parasite and the infection rate in the colony (Poinar and Yanoviak 2008). We kept the number of adult infected ants killed in the project to a minimum to avoid disrupting the parasite cycle. When possible, live ants were returned to their respective colonies after experiments.

Cephalotes atratus workers commonly accumulate around patchy resources, such as fresh bird feces, where they are conspicuous and relatively easy prey. We hypothesized that the abundance of infected ants would be disproportionately high at such stationary food items. To test this, we compared the relative abundance of infected ants at tuna baits with that of ants transiting along vines with

a Wilcoxon two-sample test (SAS Institute 2002) using the count data described above. We similarly tested the relative frequency of gaster flagging in infected ants versus normal ants that were observed during counts. Proportional data were arcsine square-root transformed before analysis (Sokal and Rohlf 1995). Other statistical tests are identified in the results.

Effects on Ants

To quantify general physical differences between infected and normal ants, we weighed freshly collected workers to the nearest 0.1 mg on an analytical balance and estimated body size by measuring head width (HW) behind the eyes (between the vertex spines) to the nearest 0.01 mm with digital calipers. We used HW as a surrogate for body size because it is often correlated with other measures (Corn 1980; Tralalon et al. 2000; but see Kaspari and Weiser 1999). We dissected the gasters of 10 infected workers to assess general changes in internal anatomy caused by the nematodes. We also collected nematode eggs from three of the ants and mounted them on microscope slides with Euparal following dehydration in an ethanol series.

To quantify the change in color of parasitized ants, we measured the percentage reflectance of the gasters of 11 infected and 11 normal *C. atratus* workers from 380 to 700 nm at 10-nm intervals with a spectroradiometer (GretagMacbeth Eye-One, Grandville, MI). We also measured the reflectance of 11 ripe *Hyeronima alchorneoides* (Euphorbiaceae) fruits present in the general area of infected colonies for comparison with the red gasters (fig. 2). Each gaster or fruit was measured more than five times and repositioned between recordings. To condense the large amount of information thus obtained, we used the median reflectance value at each wavelength across the spectrum to generate a representative profile for each individual ant or fruit. The averages of these individual profiles were plotted for comparison.

We observed that infected foragers tend to hold their gasters in an elevated position (i.e., gaster flag) almost continuously (see "Results"). We hypothesized that this behavioral change is simply biomechanical compensation for the mass added by the parasite load. To test this, we monitored the gait of seven normal workers after increasing the weight of their gasters by adhering fine sand to fingernail polish freshly painted on the gaster dorsum. The ants were weighed before and after this treatment as described above.

When handled and manipulated in the lab, the gasters of infected individuals appeared to readily detach from the body at the postpetiole junction. To quantify the breaking strength of this junction, we secured seven normal and seven infected ants to a small sheet of cardboard by in-

serting one insect pin on either side of the postpetiole. The pins crossed to create an opening too small for the gaster to pass, but large enough to avoid contact with the postpetiole. The thorax of each ant was grasped in the terminal clip of a 300-g spring scale, and tension was gradually increased along the longitudinal axis of the ant until the gaster broke free. Ants were paired a priori by body size based on HW. We used a similar technique to measure leg removal forces from seven infected and seven normal ants paired by HW. Ants were secured to the cardboard sheet with pins crossing over the thorax. One midleg was grasped midfemur in the spring scale clip, and the force at breakage of the trochanter-femur joint was recorded as described above.

To put the results of these experiments into a more natural context (i.e., to determine whether the red gaster could easily be plucked from an infected foraging ant), we measured the force required to dislodge nine normal ants from their typical foraging substrate. A thread was secured to the dorsal surface of each ant's gaster with cyanoacrylate adhesive. A spring scale was connected to the loose end of the thread, and the ant was allowed to walk freely on a twig ca. 50 cm long and 1 cm in diameter. The thread was then pulled taut and the tension gradually increased until the ant lost its grip on the substrate. We recorded the force at the time of release and measured the HW of the ant as described above.

Predation by Birds

We conducted three experiments to estimate relative predation rates on red gasters and to implicate the potential predators. In the first experiment, two similar-sized *C. atratus* workers, one infected and one normal, were secured to a twig ca. 1 cm in diameter and 30 cm long. The ants were spaced >10 cm apart, and each was held in place by a loop of fishing line passed around the twig and transversely over the thorax between the pronotal and propodeal spines. Thirteen replicates of experimental twigs were tied to vines in tree fall gaps or to branches in the forest canopy on BCI, and a green plastic colander was suspended beneath each replicate to collect any fallen ant parts. Each colander was preloaded with a gaster removed from a normal *C. atratus* to ensure that fallen ant parts were not being scavenged by other ants. The experiment was checked at least once a day until an ant was missing its gaster or had died. Six of the 13 replicate pairs ultimately were excluded from the analysis: gasters were missing from both experimental ants in two replicates, both ants died before being depredated in three replicates, and the red gaster was found in the subtending colander in one replicate.

In the second experiment, we placed various colors of

clay balls (red, black, pink, yellow, orange, gray, white, blue, green, and brown; Polyform Products, Elk Grove Village, IL) on the ends of pins (fig. 4). The clay balls were ca. 5 mm in diameter (similar to the gaster diameter of a medium-sized *C. atratus* worker) and were serially arranged along vines or tree branches at the BCI, ACTS, and CICRA field sites ($n = 27$ replicate series, all sites combined). The pins in a given linear series were separated by ca. 10 cm and inserted at an acute angle such that each clay ball was elevated 1–2 cm above the bark surface. The order of the 10 colors in each series was haphazard. The clay balls were examined for damage once per day after placement.

In the final experiment, we fed one infected and one normal *C. atratus* worker to a free-range chicken housed at CICRA for use in an unrelated research project. We collected feces deposited by the chicken immediately before and 1.5, 2.5, and 13 h after feeding it the ants. The feces were preserved in 70% ethanol and examined for the presence of ant parts and nematode eggs under $40\times$ magnification.

To quantify the proportion of bird feces gathered by the ants, we collected >300 large particulate food items from *C. atratus* foragers returning to the nest of an infected colony on BCI in May 2007. Collections were made between 0900 and 1730 hours for 0.5–2 h per day over 5 days. All bird feces encountered in these samples were dissected in 1% saline solution under $40\times$ magnification.

Results

Four of the 101 *Cephalotes atratus* colonies examined (all sites pooled) were infected: three in Panama (two on BCI and one near Camino de Cruces) and one at CICRA in Peru. Infection rates (based on red-gastered workers observed outside the nests) averaged 3.5% for the two parasitized colonies on BCI (range = 1.5%–10.0% of workers per count). In contrast, only three red-gastered foragers were found out of several thousand workers encountered in the CICRA colony, but dissection of that colony's brood showed the actual infection rate to be ca. 6% (Poinar and Yanoviak 2008). The infection rate was not determined for the colony at Camino de Cruces.

Infected ants did not accumulate disproportionately at stationary patches of food resources, as we had hypothesized. The average relative abundance of infected workers foraging at tuna baits (3.0%, $n = 10$ counts, range = 1.2%–5.0%) was similar to their abundance among workers running along vines (3.3%, range = 1.3%–5.0%, $z = 0.49$, $P = .62$).

Effects on Ants

The parasite has multiple physical and behavioral effects on the ants (table 1). The most conspicuous of these is the change in color from black to red (figs. 1, 2). Although the greatest degree of color change is restricted to the gaster, many individuals also show some reddening of the femora (fig. 1). Dissection of normal and infected workers revealed that the mechanism for the color change does not involve production of red pigmentation. Instead, the exoskeletal structure or pigment density of the gaster is altered in infected ants such that it becomes translucent amber, which, in combination with the yellowish nematode eggs held inside, causes a bright red appearance. Gaster reddening is not uniform among infected workers, and the extent of discoloration is correlated with certain behavioral changes, as described below. Reflectance profiles for the gasters of infected ants were similar in shape and intensity to those for mature *Hyeronima alchorneoides* fruits (fig. 2).

Apart from the change in color, parasitized ants consistently exhibit two other external anatomical differences from normal ants. First, infected workers are 10% smaller but 40% heavier than similar-sized uninfected ants (Poinar and Yanoviak 2008; fig. 3). This incongruous weight gain is due to the parasite load. The rate of mass increase was similar between infected and normal ants (t -test for difference in slopes; $t = 1.41$, $df = 1$, $P = .16$; fig. 3). Second, the parasite causes significant weakening of the postpetiole-gaster junction. The average (\pm SE) tensile force required to remove the gaster from an infected ant is one-fourteenth (18 ± 3.9 g) that for a healthy ant (254 ± 28.2 g; paired $t = 9.66$, $df = 6$, $P < .0001$). The ants adhere to twig surfaces with 15 ± 1.2 g of force; thus, the parasite-laden gaster may be plucked from a forager without removing the ant from its natural substrate ($t = 0.81$, $df = 14$, $P = .43$). This is not true for healthy ants.

Table 1: Principal effects of tetradonematid infection on *Cephalotes atratus* workers

Target characteristic	Appearance
Physical effect:	
Gaster	Red, full of eggs
Body size (HW)	10% smaller
Mass	40% heavier
Gaster-postpetiole junction	93% weaker
Internal organs	Nerve cord damage
Behavioral effect:	
Gaster flagging	Virtually constant
Gait	Erect and unstable
Alarm/defensive pheromone production	Little or none
General activity	Sluggish

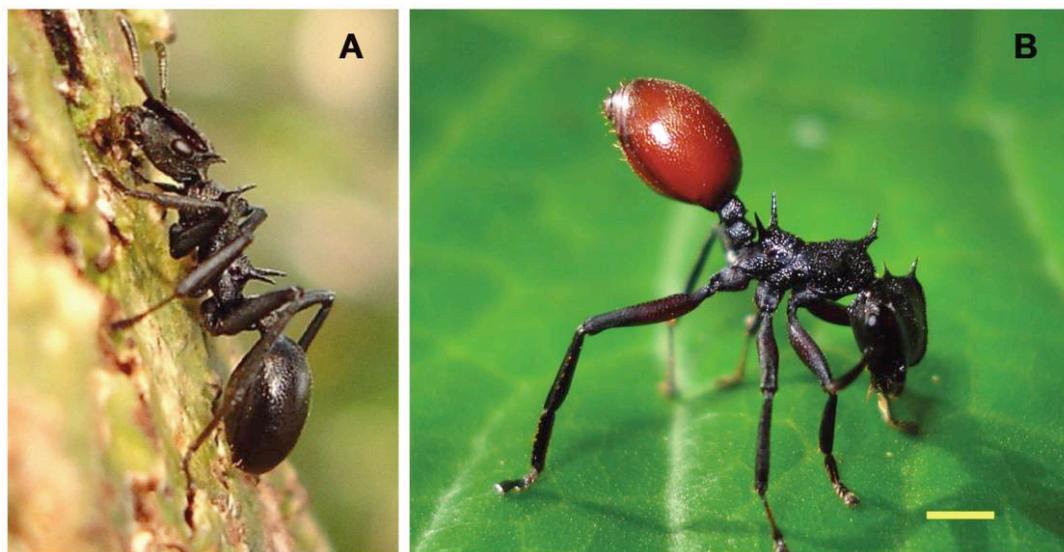


Figure 1: Normal (A) and infected (B) *Cephalotes atratus* workers. Scale bar \approx 2 mm.

This exoskeletal weakening is restricted to the postpetiole-gaster junction; there was no difference in the force required to remove legs from normal (159 ± 10.6 g) and infected (143 ± 13.0 g) workers (paired $t = 1.44$, $df = 6$, $P = .23$).

Dissection of infected and uninfected ants revealed that the physical effects of the parasite extend to their internal anatomy. The gastrointestinal tract and Malpighian tubules of infected ants were completely intact, but the portion of the ventral nerve cord located in the gasters of infected ants consistently appeared atrophied and dark in color. In most cases, it was clearly visible as a dark semicircle or coil under the translucent gastral sternites. Further dissection showed that the parasites are not found in other regions of the ant apart from the gaster.

Nematode infection has several behavioral effects on the ants in addition to the physical effects described above (table 1). Most conspicuous among these is nearly constant gaster flagging (frequency among infected workers = 94.5%, range = 86%–100%, $n = 10$ counts) relative to normal workers (3.9%, range = 2.7%–5.8%; Wilcoxon two-sample test, $z = 3.77$, $P = .0002$). Adding sand to the gasters of normal workers increased their mass by an average of 47% (range = 7%–120%) and caused minor instability when walking, but did not stimulate continuous gaster flagging or otherwise alter their behaviors in ways resembling infected workers.

Changes in the appearance and behavior of the ants are associated with each other and linked to development of the nematodes. Specifically, individuals with the greatest amount of red coloration are less aggressive (they do not

bite when handled) and produce no detectable alarm pheromones when intentionally disturbed. (Like many *Cephalotes* species, *C. atratus* produces alarm chemicals that are distasteful and have a strong odor readily detected by humans.) They also are the most sluggish and the most unstable among infected ants. In contrast, ants with incomplete reddening of the gaster occasionally release alarm pheromones when disturbed, show typical levels of aggression and defense, and have a normal gait apart from continuous gaster flagging. The extent and intensity of redness increases as the nematode embryos develop into first-instar worms (see Poinar and Yanoviak 2008).

Predation by Birds

Gaster removal rates were higher for infected than for normal ants when they were presented in tethered pairs in the field on BCI (binomial test, $P = .055$). The missing gasters in these replicates were not found in the respective colanders, nor were the “seed” gasters removed from the colanders. In the second predation experiment, red and pink clay balls (combined) either were attacked by birds or were missing more often than the other colors combined (G -test; $G = 4.61$, $df = 1$, $P = .036$). Bird attacks were distinguishable from damage caused by other animals because of the V-shaped impressions left in the clay (fig. 4). Finally, hundreds of intact tetradonematid eggs appeared in the chicken feces between 2.5 and 13 h after the chicken was fed an infected ant.

The majority of the solid material carried by foraging *C. atratus* consisted of bird feces (68%), followed by dead

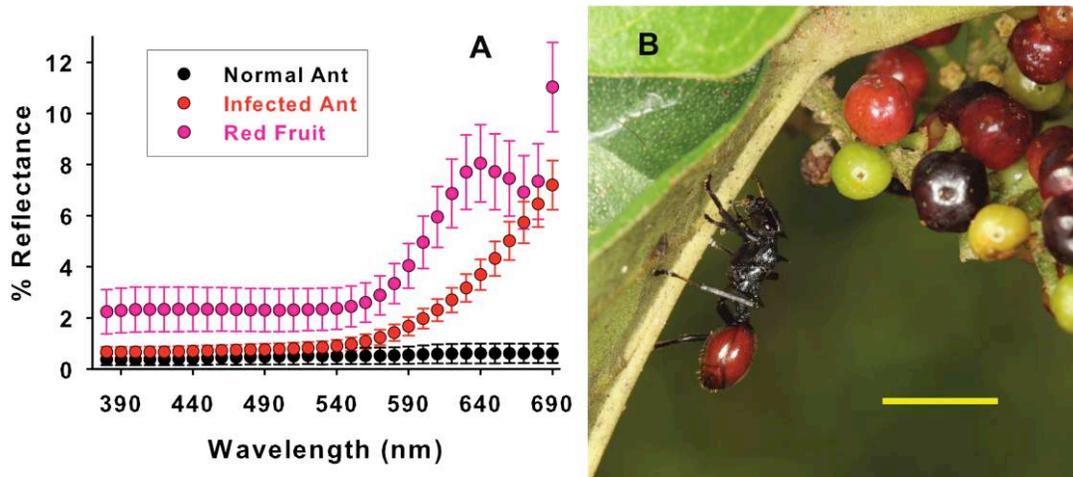


Figure 2: A, Reflectance profiles of gasters of normal and infected *Cephalotes atratus* workers and of ripe *Hyeronima alchorneoides* fruits. For each series, $n = 11$ ants or fruits, and each point is the average ($\pm 95\%$ confidence interval) of the median values obtained from multiple recordings per ant or fruit. B, An infected ant is shown on an infructescence of *H. alchorneoides* for comparison. Scale bar ≈ 1 cm.

insects and insect parts (24%), brood (eggs and larvae; 8%), and unidentifiable material (<1%). We found one nematode egg containing a live worm (although not confirmed to be a tetradonematid) and several other living microscopic worms and parasite eggs in the bird feces carried by the ants.

Discussion

Here we show that a parasitic tetradonematid nematode has multiple physical and behavioral effects on its terminal host, the common Neotropical arboreal ant *Cephalotes atratus*, that likely facilitate its transmission to the same colony and to new colonies via frugivorous birds. Most conspicuous among these effects is the striking change in gaster color from black to red, which earned infected workers separate taxonomic status in the late nineteenth century (var. *rufiventris* Emery; de Andrade and Baroni Urbani 1999; Poinar and Yanoviak 2008).

Our results suggest the following hypothesized and possibly unique life cycle for this new nematode species. A frugivorous bird mistakenly consumes an infected gaster and deposits nematode eggs in its feces while perched in a tree crown. The feces are collected by a foraging *C. atratus* (Corn 1980; this study), but the filter-like proventriculus of the ant's gastrointestinal tract prevents passage of nematodes to its midgut (Baroni Urbani and de Andrade 1997; Roche and Wheeler 1997). Instead, transmission to new ants occurs when the nematode-laden feces are fed to larvae by workers inside the nest. The nematodes develop within the ant larvae, partly stunting their growth. Adult

worms migrate to the pupal gaster and mate within the callow worker (Poinar and Yanoviak 2008). Shortly after eclosion, the parasitized adult ant has a black gaster and mainly tends brood, rarely leaving the nest (Corn 1980). Hundreds of developing nematode embryos within the gravid female worms cause increased reddening and modified behavior, probably by sequestering food and exoskeletal compounds from the adult ant. Just as a fruit reaches peak color when its seeds are ready for dispersal, the gaster reaches peak redness as the nematode eggs are

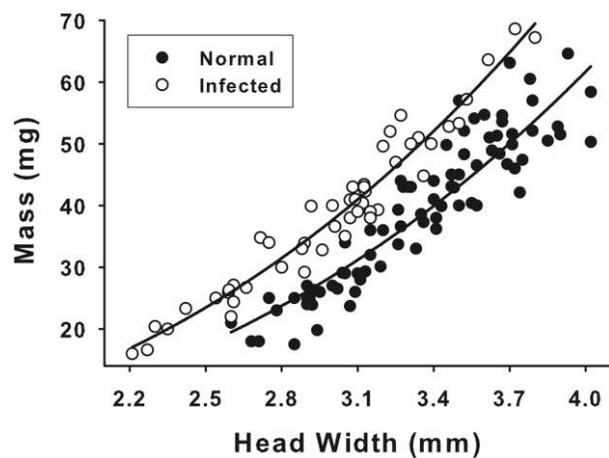


Figure 3: Head width (HW) versus mass regressions for normal ($n = 84$) and infected ($n = 49$) *Cephalotes atratus* workers. The slopes of the two regressions do not differ. Infected equation: $\text{mass} = 2.19\text{HW}^{2.59}$; normal equation: $\text{mass} = 1.51\text{HW}^{2.68}$.

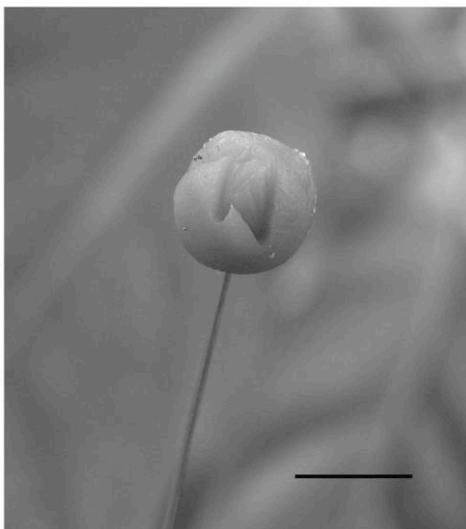


Figure 4: Clay ball perched on an insect pin used in predation experiments. Bird attacks are indicated by the V-shaped impressions. Scale bar \approx 5 mm.

most infective. These changes coincide with temporal polyethism in *C. atratus*; the worker ant switches from brood care inside the nest to foraging as it ages (Corn 1980). The gaster is consumed by a frugivorous bird while the ant is outside the nest, and the parasitic life cycle repeats.

Despite hundreds of hours of observing and recording bird and ant behaviors in tree crowns, we lack direct observation of bird predation on either infected or healthy *C. atratus*. However, all evidence gathered to date indicates that transmission by duped frugivorous birds is the most parsimonious explanation for the changes induced in *C. atratus* by the parasite. Among tropical ants, *C. atratus* workers are particularly well suited to this mimicry system because of their large size, conspicuous spherical gasters, diurnal activity, arboreal habits, and extensive foraging on bird feces (Corn 1980; this study). Although they will occasionally forage on or near the ground (especially in disturbed sites; Weber 1957; Corn 1980), *C. atratus* are primarily arboreal, and the redness of the gasters is most intense (to the human observer) in direct sunlight. Thus, involvement of an arboreal paratenic host (i.e., an intermediate host required for completion of the parasite's life cycle but in which no development of the parasite occurs) is the simplest scenario. There are many logical candidates for paratenic hosts among frugivorous birds, including several species of honeycreepers, tanagers, and small tyrannid flycatchers (D. Robinson, personal communication; also see Hosner 2005). Collectively, the reduced alarm defenses, the weakened gaster-postpetiole junction, and the

general sluggishness caused by the parasite should facilitate consumption of an infected gaster by a duped frugivore.

Two alternatives to the fruit mimicry scenario must be considered. First, it is possible that all of the described changes are irrelevant to transmission (i.e., no specific paratenic hosts are involved and reinfection occurs within the nest), as might occur if carcasses of parasitized ants are fed to larvae. However, this pathway provides no mechanism for infection of new *C. atratus* colonies unless (a) foundress queens carry the parasite or (b) infected workers invade, or are enslaved by, other colonies (Breed et al. 1999). We did not find nematodes in either male or queen pupae. An infected foundress likely would have very low survival and fecundity, and queen replacement is not known for *C. atratus*. Colonies of this species often are very large (Weber 1957; Corn 1980) and may persist for many years, if not decades. Thus, if transmission occurs via enslavement, we would expect infected *C. atratus* colonies to be clumped in space. We believe that the absence of a paratenic host is a very unlikely scenario for this parasite, but more data are needed to adequately assess this possibility.

The second alternative explanation to fruit mimicry is that the parasite causes increased conspicuousness of infected ants to generalist insectivores. This hypothesis requires a priori that healthy hosts are regularly consumed by predators. Despite being very conspicuous (large, black, diurnal ants that forage in large numbers, often against light-colored tree surfaces; see Yanoviak and Dudley 2006), records of insectivorous vertebrates feeding on *C. atratus* are very few (de Andrade and Baroni Urbani 1999). Relative to their abundance and conspicuousness, ants in general do not represent a large fraction of the prey consumed by tropical insectivorous birds (Chapman and Rosenberg 1991), and there are no published records of bird predation on *C. atratus*. Our field observations indicate that *C. atratus* workers are generally ignored by insectivorous birds, probably because (like many other *Cephalotes* species) they are well defended with spines, hard exoskeletons, and distasteful pheromones (see de Andrade and Baroni Urbani 1999). A significant reduction in these defenses is not required by the increased conspicuousness hypothesis but must accompany fruit mimicry for it to be effective. Our results show that this is the case in parasitized *C. atratus*.

Whereas birds do not regularly feed on *C. atratus*, other predators, such as anteaters and lizards, could support the increased conspicuousness scenario (de Andrade and Baroni Urbani 1999). Arboreal ants are a major component in the diets of tropidurid lizards (Vitt and Zani 1996), and *Uracentron* feces collected at the ACTS site contained *Cephalotes* spp. (S. P. Yanoviak, personal observation). However, the patchy nature of *C. atratus* distribution in forests requires a reliable mechanism of parasite trans-

mission to new colonies. If this is accomplished by a vertebrate paratenic host, birds and their feces are the most efficient vehicles, given the natural history of *C. atratus*. It is unlikely that lizards would disperse over great distances or with sufficient frequency to infect new *C. atratus* colonies. Moreover, unlike bird feces, feces of anteaters and arboreal lizards are relatively dry and fall to the ground, where they are likely to be consumed by litter ants (Kaspari 1993) long before their discovery by *C. atratus* foragers. Finally, regardless of the predator, red coloration (especially in combination with black) is generally aposematic in insects (Chapman 1998); thus, we would expect lower predation by generalist insectivores on infected *C. atratus*.

Physiological mechanisms underlying the coordinated physical and behavioral effects of the tetradonematid on *C. atratus* are unknown and deserve further investigation. Parasite-induced changes in appearance occur in other ant taxa, although none suggests fruit mimicry. When such color changes occur, they generally affect the whole body, not specific parts as in *C. atratus* (Schmid-Hempel 1998). In the few examples in which pale coloration is restricted to the gaster, the effect is a by-product of gaster distention, not altered pigmentation (e.g., Carney 1969). No color change was observed in other ants infected with tetradonematids (Nickle and Jouvenaz 1987) or in a *Cephalotes minutus* infected by a mermithid nematode (de Andrade and Baroni Urbani 1999). The color change in *C. atratus* may result in part from cuticular thinning (Crosland 1988). This is supported by the fact that thinner areas of exoskeleton on the frontal carinae of *C. atratus* are translucent orange and that another exoskeletal structure—the gaster-postpetiole junction—is significantly weakened by the infection. Alternatively, the developing nematodes may specifically sequester pigment compounds from the adjacent exoskeleton without significantly compromising its structural integrity.

The 10% size reduction we observed in *C. atratus* is comparable to measured HW changes in other ants that are parasitized by cestodes and nematodes during larval development (Passera 1976; Trabalon et al. 2000). *Cephalotes atratus* workers show considerable within-colony size variation that is essentially continuous (Corn 1980). Given the substantial overlap in the size distribution of infected and normal workers (fig. 3), the ecological consequences of a 10% size reduction in 5% of the workers are probably irrelevant at the colony level. A number of factors could cause the smaller body size in infected workers, including malnutrition, slower growth rates, or hormonal aberrations.

The continuous gaster flagging and erect stance of parasitized *C. atratus* workers have not been documented for other ants infected with parasites. Our observations sug-

gest that these behavioral effects are partly caused by damage to the ventral nerve cord in the gaster, perhaps via mechanical pressure imposed by the parasite mass. Anterior progression of this damage as the parasites develop in the adult ant may explain the increased sluggishness and unstable stance of workers during the latter stages of infection.

Two-host cycles like the one described here are known for other nematodes, and this strategy is effective for parasites of sedentary, central-place foragers such as ants because the nematode can complete its entire life cycle near the nest (Schmid-Hempel 1998). Other nematode parasites of ants have effects somewhat similar to those described here, including reduced defensive behaviors combined with lethargy (e.g., Gösswald 1930). However, we know of none that combines a change in defense-based palatability with fruit mimicry, nor do we know of similarly spectacular cases in which an insect mimic and its model occupy separate biological kingdoms (but see Roy 1993 for a striking plant-fungus relationship). Although it is likely that the extensive collection of bird feces by *C. atratus* played a significant role in the evolution of this association, understanding the interplay of coordinated effects on the ant awaits further study of their mechanisms. We hope that this discovery promotes additional research into other potential examples of facilitation of parasite transmission via mimicry.

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Literature Cited

- Baroni Urbani, C., and M. L. de Andrade. 1997. Pollen eating, storing, and spitting by ants. *Naturwissenschaften* 84:256–258.
- Breed, M. D., T. P. McGlynn, E. Stocker, and A. N. Klein. 1999. Thief workers and variation in nestmate recognition behavior in a ponerine ant, *Ectatomma ruidum*. *Insectes Sociaux* 46:327–333.
- Carney, W. P. 1969. Behavioral and morphological changes in car-

- penter ants harboring microcoeliid metacercariae. *American Midland Naturalist* 82:605–611.
- . 1970. *Brachylecithum mosquensis*: infections in vertebrate, molluscan and arthropod hosts. *Transactions of the American Microscopical Society* 89:233–250.
- Chapman, A., and K. V. Rosenberg. 1991. Diets of four sympatric Amazonian woodcreepers (Dendrocolaptidae). *Condor* 93:904–915.
- Chapman, R. F. 1998. *The insects: structure and function*. 4th ed. Cambridge University Press, Cambridge.
- 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.
- Curio, E. 1976. *The ethology of predation*. Springer, Berlin.
- de Andrade, M. L., and C. Baroni Urbani. 1999. Diversity and adaptation in the ant genus *Cephalotes*, past and present. *Stuttgarter Beiträge zur Naturkunde, Serie B (Geologie und Paläontologie)*, no. 271. Stuttgart Museum of Natural History, Stuttgart.
- Gösswald, K. 1930. Weitere Beiträge zur Verbreitung der Mermithiden bei Ameisen. *Zoologischer Anzeiger* 90:13–27.
- Heinze, J., O. Rüppell, S. Foitzik, and A. Buschinger. 1998. First records of *Leptothorax rugatulus* (Hymenoptera: Formicidae) with cysticercoids of tapeworms (Cestoda: Dilepididae) from the southwestern United States. *Florida Entomologist* 81:122–125.
- Holmes, J. C., and W. M. Bethel. 1972. Modification of intermediate host behavior by parasites. Pages 123–149 in E. U. Canning and C. A. Wright, eds. *Behavioral aspects of parasite transmission*. Academic Press, London.
- Hosner, P. A. 2005. Regurgitated mistletoe seeds in the nest of the yellow-crowned tyrannulet (*Tyrannulus elatus*). *Wilson Bulletin* 117:319–321.
- Hurd, H. 1990. Physiological and behavioral interactions between parasites and invertebrate hosts. *Advances in Parasitology* 29:271–318.
- Kaspari, M. 1993. Removal of seeds from Neotropical frugivore droppings: ant responses to seed number. *Oecologia (Berlin)* 95:81–88.
- Kaspari, M., and M. D. Weiser. 1999. The size-grain hypothesis and interspecific scaling in ants. *Functional Ecology* 13:530–538.
- Leigh, E. G., Jr., A. S. Rand, and D. M. Windsor, eds. 1996. *The ecology of a tropical forest*. 2nd ed. Smithsonian Institution, Washington, DC.
- Lindström, L., R. V. Alatalo, A. Lyytinen, and J. Mappes. 2001. Strong antiapostatic selection against novel rare aposematic prey. *Proceedings of the National Academy of Sciences of the USA* 98:9181–9184.
- Madigosky, S. R., and I. Vatnick. 2000. Microclimatic characteristics of a primary tropical Amazonian rain forest, ACEER, Iquitos, Peru. *Selbyana* 21:165–172.
- Mäki, S., R. Kalliola, and K. Vuorinen. 2001. Road construction in the Peruvian Amazon: causes and consequences. *Environmental Conservation* 28:199–214.
- Moore, J. 1995. The behavior of parasitized animals. *BioScience* 45:89–96.
- . 2002. *Parasites and the behavior of animals*. Oxford University Press, Oxford.
- Muir, D. A. 1954. Ants *Myrmica rubra* L. and *M. scabrinodis* Nyl. as intermediate hosts of a cestode. *Nature* 173:688–689.
- Nickle, W. R., and D. P. Jouvenaz. 1987. *Tetradonema solenopsis* n. sp. (Nematoda: Tetradonematidae) parasitic on the red imported fire ant *Solenopsis invicta* Buren from Brazil. *Journal of Nematology* 19:311–313.
- Passera, L. 1976. Origine des intercastes dans les sociétés de *Pheidole pallidula* (Nyl.) (Hymenoptera, Formicidae) parasitées par *Mermis* sp. (Nematoda, Mermithidae). *Insectes Sociaux* 23:559–575.
- Peloquin, J. J., and E. G. Platzer. 1993. Control of root gnats (Scuridae: Diptera) by *Tetradonema plicans* Hungerford (Tetradonematidae: Nematoda) produced by a novel culture method. *Journal of Invertebrate Pathology* 62:79–86.
- Poinar, G., Jr., and S. P. Yanoviak. 2008. *Myrmeconema neotropicum* n. g., n. sp., a new tetradonematid nematode parasitising South American populations of *Cephalotes atratus* (Hymenoptera: Formicidae), with the discovery of an apparent parasite-induced host morph. *Systematic Parasitology* 69:145–153.
- Roche, R. K., and D. E. Wheeler. 1997. Morphological specializations of the digestive tract of *Zacryptocerus rohweri* (Hymenoptera: Formicidae). *Journal of Morphology* 234:253–262.
- Roy, B. A. 1993. Floral mimicry by a plant pathogen. *Nature* 362:56–58.
- SAS Institute. 2002. SAS OnlineDoc 9. SAS Institute, Cary, NC.
- Schmid-Hempel, P. 1998. *Parasites in social insects*. Princeton University Press, Princeton, NJ.
- Sheiman, I. M., M. F. Shkutin, N. B. Terenina, and M. K. S. Gustafsson. 2006. A behavioral study of the beetle *Tenebrio molitor* infected with cysticercoids of the rat tapeworm *Hymenolepis diminuta*. *Naturwissenschaften* 93:305–308.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry*. 3rd ed. W. H. Freeman, New York.
- Tang, Y., R. Killick-Kendrick, and W. M. Hominick. 1997. Life cycle of *Didilia ooglypta* (Nematoda: Tetradonematidae), a parasite of phlebotomine sandflies of Afghanistan. *Nematologica* 43:491–503.
- Trabalon, M., L. Plateaux, L. Péru, A.-G. Bagnères, and N. Hartmann. 2000. Modification of morphological characters and cuticular compounds in worker ants *Leptothorax nylanderii* induced by endoparasites *Anomotaenia brevis*. *Journal of Insect Physiology* 46:169–178.
- Vásquez Martínez, R. 1997. Flórmula de las reservas biológicas de Iquitos, Perú. *Monographs in systematic botany*, vol. 63. Missouri Botanical Garden, St. Louis.
- Vitt, L. J., and P. A. Zani. 1996. Ecology of the elusive tropical lizard *Tropidurus* [= *Uracentron*] *flaviceps* (Tropiduridae) in lowland rain forest of Ecuador. *Herpetologica* 52:121–132.
- Weber, N. A. 1957. The nest of an anomalous colony of the arboreal ant *Cephalotes atratus*. *Psyche* 64:60–69.
- Wheeler, W. M. 1907. The polymorphism of ants, with an account of some singular abnormalities due to parasitism. *Bulletin of the American Museum of Natural History* 23:1–93.
- Yanoviak, S. P., and R. Dudley. 2006. The role of visual cues in directed aerial descent of *Cephalotes atratus* workers (Hymenoptera: Formicidae). *Journal of Experimental Biology* 209:1777–1783.
- Zervos, S. 1980. *Bispiculum inaequale* n. gen. & sp. (Nematoda: Tetradonematidae) from New Zealand wood-boring beetles (Curculionidae: Platypodinae). *New Zealand Journal of Zoology* 7:155–164.