

# Mimicry and Eavesdropping Enable a New Form of Social Parasitism in Ants

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**ABSTRACT:** Social parasitism is defined by the exploitation of the social mechanisms of one society by another whole society. Here, we use quantitative ecological data and experiments to identify the components of a new form of social parasitism by the recently discovered “mirror turtle ant,” *Cephalotes specularis*. We show that *C. specularis* workers visually mimic and actively avoid contact with foragers of the hyperaggressive host ant *Crematogaster ampla*, allowing them to move freely in the extensive and otherwise defended foraging networks of host colonies. Workers from parasite colonies have immediate access to these networks by nesting exclusively within host territories, and 89% of all potential host territories were parasitized. Inside the network, parasite workers eavesdrop on the host’s trail pheromones to locate and exploit food resources that are defended by the host to the exclusion of all other ants. Experiments demonstrated the unprecedented capacity of the parasite for superior foraging performance on its host’s pheromone trails than on trails of its own. Considered together, the apparent Batesian-Wallacian mimicry, pheromone-based interceptive eavesdropping, kleptoparasitism, and xenobiotic nesting ecology displayed by *C. specularis* within the territory and foraging network of a host ant represents a novel adaptive syndrome for social exploitation.

**Keywords:** social parasitism, eavesdropping, Batesian-Wallacian mimicry, xenobiosis, turtle ants, *Cephalotes*.

## Introduction

Among the many varied forms of parasitism (reviewed in Price 1980; Combes 2001; Hatcher and Dunn 2011), social parasitism is a special case where the social mechanisms of one society are exploited by another whole society (Buschinger 1986, 2009; Tizo-Pedroso and Del-Claro 2014). One society then lives inside the social system of the other, deriving key resources from the host. Understanding how and why social parasitism evolves remains

particularly challenging, because the complex parasitic interactions are typically hidden inside the host’s concealed and guarded nests (reviewed in Huang and Dornhaus 2008; Buschinger 2009; Tizo-Pedroso and Del-Claro 2014). Moreover, opportunities to understand the early transition from free living to a socially parasitic lifestyle are especially scarce, because the derived social parasites in many of the most accessible and intensively studied systems have lost key aspects of their ancestral free-living biology (Huang and Dornhaus 2008; Buschinger 2009). New examples of social parasitism that may help overcome these barriers are therefore of considerable value.

Existing examples of social parasitism can be characterized into a number of broad classes, especially in the ants where social parasitism is widespread. These types, in order of increasing dependency of the parasite on the host, are xenobiosis, temporary parasitism, permanent parasitism with dulosis (also known as slavery), and inquilinism (Buschinger 2009). Most research attention has been given to the last three classes, which involve dependencies on closely related hosts for brood rearing within a mixed nest (reviewed in Buschinger 1986, 2009; Brandt et al. 2005a; Huang and Dornhaus 2008). Xenobiosis, in contrast, typically involves parasitism of food or shelter resources owned by a distantly related species, with host and parasite caring for their own brood in different nests or nesting chambers (Huang and Dornhaus 2008; Buschinger 2009). Interactions that fall into the broad category of xenobiosis are therefore a resource-based social parasitism between a host and a parasite that has typically retained much of its ancestral free-living biology. Despite the potential value of such interactions for understanding the transition from free living to social parasitism, resource-based social parasitism in ants has received limited research attention (Huang and Dornhaus 2008; Buschinger 2009). Moreover, known examples where the parasite-host interactions are

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accessible, and therefore experimentally tractable, are few and far between.

In this article, we identify a new form of resource-based social parasitism between *Cephalotes specularis*, a recently discovered and described turtle ant species (Brandão et al. 2014), and the host ant *Crematogaster ampla*. Like many arboreal *Crematogaster* species, *C. ampla* is a large-colony, highly aggressive, and territorial species with conspicuous foraging networks. Nevertheless, we first discovered *C. specularis* foragers feeding at food resources defended by *C. ampla* workers and running freely in *C. ampla* foraging trails. Moreover, the *C. specularis* workers displayed a highly atypical body posture for turtle ants that made them hard to distinguish from the *Crematogaster* foragers. These natural history discoveries suggested a new type of resource-based social parasitism operating in the foraging arena, not within the host's nest.

Building from our initial observations, we combine quantitative ecological data and experimental studies to provide the first characterization of the different components of the parasite-host interaction. Specifically, we present data on the parasite's foraging strategy and components of the mimicry system, as well as parasite-host ecological co-occurrence patterns, nesting ecology, and colony sizes. We then use the high level of experimental tractability of this system to test a key hypothesis derived from our characterization: that the parasite's foraging performance is enhanced by the capacity to "eavesdrop" on the host's pheromone-based foraging trails. Eavesdropping is defined as the exploitation of signaled information intended for another receiver (Peake 2005). Examples of eavesdropping on chemically signaled information are relatively rare (reviewed in Haynes and Yeagan 1999; Peake 2005), even for the pheromone-based foraging trails of ants (e.g., Adams 1990; Menzel et al. 2010). The apparent capacity of *C. specularis* to exploit the pheromone-based trails of *C. ampla* is therefore central to understanding the evolution of this novel parasite-host interaction.

## Methods

### *Turtle Ant Biology and Focal Species*

The turtle ants (genus *Cephalotes*) are a diverse lineage of arboreal ants, with 118 valid extant species currently recognized (de Andrade and Baroni Urbani 1999; Brandão et al. 2014; Bolton 2014). The group is strictly New World in distribution, and most species are found in the Neotropics (de Andrade and Baroni Urbani 1999). All species studied to date nest obligately in plant stems, with most nesting in the abandoned cavities of wood-boring beetles that they find in the environment (Creighton 1963; Creighton and Nutting 1965; de Andrade and Baroni Urbani

1999; Powell 2008; Powell 2009). The ants have limited ability to modify these hard wood cavities, so additional cavities are found and inhabited as a colony grows (i.e., obligate polydomy past the initial colony founding stage; Powell 2009; Powell and Dornhaus 2013). Morphologically, the group is characterized by a heavily thickened cuticle that often includes shell-like cuticular projections in workers (see de Andrade and Baroni Urbani 1999), giving the group its common name. These morphological structures protect workers from biting attacks by other ants as they forage in the highly competitive canopy environment (Coyle 1966). Most species also have soldiers (de Andrade and Baroni Urbani 1999), which have a specialized head that is used to physically block the entrances of nesting cavities (Creighton and Gregg 1954; Creighton 1963; Powell 2008, 2009).

The focal turtle ant species for this study has been described as *Cephalotes specularis* (Brandão et al. 2014), or the "mirror turtle ant," because it has a highly reflective terminal body segment (gaster) and "mirrors" the appearance and behavior of its ant host. The host ant species was identified as *Crematogaster ampla* (determined by J. T. Longino).

### *Study Site and Turtle Ant Diversity Survey*

Fieldwork was conducted at the reserve of Clube Caça e Pesca Itororó, Uberlândia, Brazil. This 400-ha reserve is dominated by native cerrado vegetation categorized as woodland savanna (see Alves-Silva and Del-Claro 2013 for more detailed site description). Typical for cerrado habitat, canopy height is approximately 3–6 m and therefore highly accessible.

A turtle ant diversity survey was conducted for the study site, resulting in both the discovery of *C. specularis* and its interactions with *C. ampla*, and a quantitative assessment of the relative abundance of *C. specularis* within the turtle ant community. Turtle ant colonies were located at random by baiting trees on nonoverlapping, 5 × 50-m transects over a 13-month period (following Powell 2008). Vertebrate urine was used as the bait, because it is a natural food source for turtle ants and prompts strong colonywide recruitment (Powell 2008, 2009). Each tree was visually inspected for the presence of turtle ant foragers 15–25 min after bait was applied. This inspection period coincides with the highest forager activity at the baits. Specimens were taken for all turtle ant species found foraging on each baited tree. Ants of a particular species seen foraging on a baited tree, or across a continuous patch of trees, were assumed to be from one colony of that species. This assumption is well supported by detailed studies of turtle ant colony structure in the cerrado (Powell 2009; this study; S. Powell, unpublished data for an additional 15

species). The species identity of all turtle ant voucher specimens was determined using the keys and species descriptions of de Andrade and Baroni Urbani (1999).

#### *Parasite-Host Co-Occurrence and Parasite Foraging Strategy*

The territories of *C. ampla* colonies were sampled to assess the frequency with which *C. specularis* co-occurred with its host. An additional 38 *C. ampla* colonies were located by walking long, nonintersecting transects through the study site. After locating a tree with *C. ampla*, the colony's territory was initially identified as all trees connected to the initial tree by a continuous network of conspicuous foraging trails populated by *C. ampla* workers. The single-colony nature of each putative territory was tested by conducting pairwise aggression tests between foragers taken from different locations (following Tschinkel et al. 1995). Tests between workers from different trees within the same connected trail network always yielded amicable interactions, indicating membership of the same colony, whereas tests between foragers from different connected trail networks resulted in immediate fighting. All visually identified single-colony territories were upheld by these tests. The presence of *C. specularis* was assessed by baiting on all trees within a *C. ampla* territory and monitoring the activity at the baits in sequential observation bouts on a trap-lining loop, until *C. ampla* recruitment substantially declined (~25 min). Observation bouts were standardized to 45 s, allowing multiple observations at each bait before *C. ampla* recruitment declined. In addition to recording the presence of *C. specularis* workers, all baits were monitored for additional ant species. This baiting procedure also facilitated further observations on the foraging strategy and related behaviors of *C. specularis*.

#### *Nesting Ecology and Colony Size*

For three *C. specularis* colonies, workers were tracked from attended baits (above) back to their nests to address nesting ecology and colony size. The initial baits were supplemented as needed to maximize recruitment and tracking. Aggression tests between workers from different nests were used to determine colony membership, because conspecific turtle ant workers from different colonies fight vigorously (following Powell 2009; Powell and Dornhaus 2013). Preliminary tests also established that *C. specularis* workers from different *C. ampla* territories fight. Once all nests of the three identified colonies had been located, they were collected (following Powell 2009) to confirm that *C. specularis* nests independently of *C. ampla*, to assess colony composition, and for use in subsequent lab experiments. On the day of collection, all nest entrances were

sealed before 7 a.m., which is 2 h before *C. specularis* colonies start foraging, and the branches housing the nests were removed and transported to the lab within 2 h. These collected colonies were also used in the description of *C. specularis* (Brandão et al. 2014).

#### *Eavesdropping and Foraging Efficiency Experiment*

Laboratory experiments were used to test the hypothesis that *C. specularis* foraging performance is enhanced by the capacity to “eavesdrop” on the *C. ampla*'s pheromone-based foraging trails. For these experiments, each *C. specularis* colony was held in a 30 × 20 × 8-cm tray with nongrip Fluon coating on the walls and given enough artificial nests to accommodate all colony members. Artificial nests were made from a 7 × 4 × 0.5-cm cavity cut in foam and sandwiched between two glass slides. In each experimental trial, two paths were arranged in a V configuration that bridged from one point in the nesting tray to identical food sources on two platforms outside of the tray. Each colony was tested with 10 trials of each of two different pairs of paths. The first pairing offered a choice between a path that had been used previously by *C. ampla* and an unused control path. The second pairing offered a choice between a path that had been used previously by *C. ampla* and a path used previously by the focal *C. specularis* colony. Within each set of 10 trials per colony, the left/right positioning was balanced, with the order randomized. Each path was used in only one trial, and a card barrier was erected to block line-of-site between paths. Paths were 5 cm × 100 cm and made from rigid art board. Paths that had been used previously by either *C. ampla* or the focal *C. specularis* colony were in place as foraging routes for at least 24 h before being extracted for use in experimental trials. The paths used by *C. ampla* were conditioned using a large *C. ampla* colony fragment kept in the laboratory. A 5-min gap was taken between extracting a path and its use in a trial. Within each paired trial, the time until food discovery along the paths was recorded. Forager traffic on the paths at 10, 15, 20, and 25 min after the trial started was also recorded and used to calculate mean forager traffic on a path, as a measure of sustained foraging activity.

## Results and Discussion

### *Foraging Ecology and Mimicry*

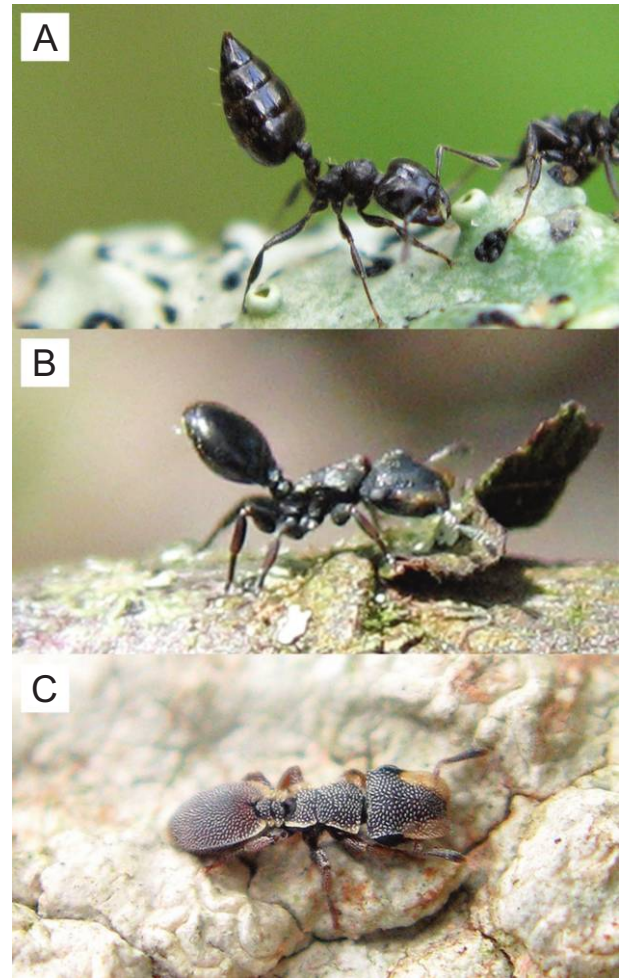
*Cephalotes specularis* workers were seen foraging only at baits dominated by *Crematogaster ampla*, or running in the dense foraging-trail traffic of *C. ampla* colonies. This spanned observations made during the initial turtle ant diversity survey, across a total of 41 *C. specularis* colonies



and 192 intensively checked baiting locations set across 38 *C. ampla* territories (further details below). No other ant species were recorded at any bait dominated by *C. ampla*, including the 192 intensively monitored baits. This is remarkable because cerrado trees typically have diverse arboreal ant assemblages, with as many as 20 species co-occurring on a single tree (Powell et al. 2011). Other ant species, including other turtle ants, were seen at territory borders, where they were chased by *C. ampla* workers and often seized and dismembered.

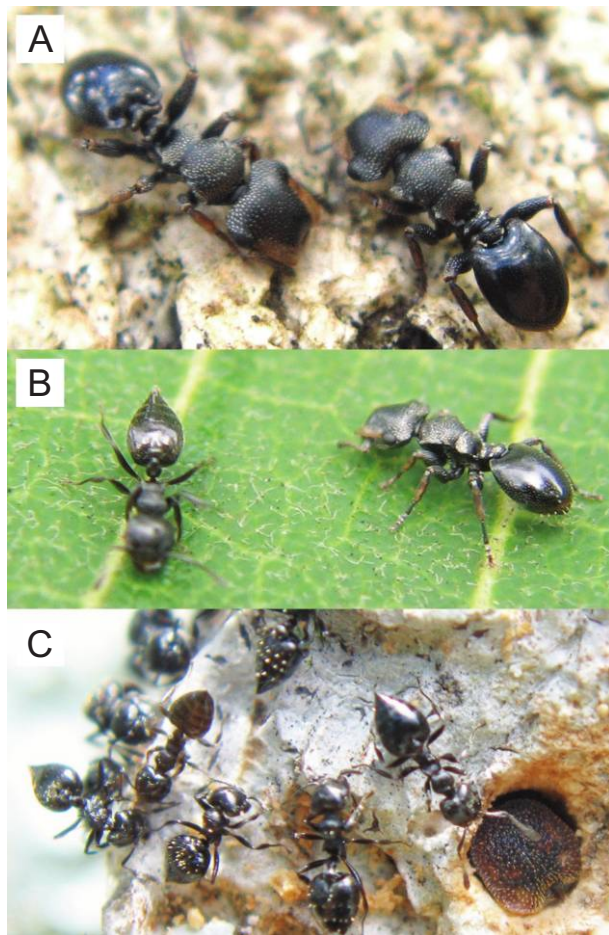
*Cephalotes specularis* workers always arrived at the baits within the *C. ampla* trail traffic, mimicking the speed and stereotyped stilt-leg posture and raised gaster of *C. ampla* (fig. 1). It is important to note that the posture mimicry is apparent in profile view, the perspective seen by the host, and is not very visible from the likely overhead view of a vertebrate predator. The visual similarity was enhanced by the matching all-black body coloration, mirror-like reflective gaster (terminal body section), and body size (figs. 1, 2). The matching black coloration and reflective gaster of *C. specularis* workers is particularly significant in the context of the other female castes of this species and its close relatives. *Cephalotes specularis* soldiers and gynes both have strikingly different coloration, including a gaster with conspicuous yellow spots, thicker hairs, and a non-reflective microsculptured surface (Brandão et al. 2014). In addition, the conspicuous reflective gaster of *C. specularis* workers is achieved in part by the significant reduction and loss of hairs on the gaster, not seen to this degree in close relatives in the combined *febrigilbruchi* clade to which *C. specularis* belongs (de Andrade and Baroni Urbani 1999; Brandão et al. 2014; *C. specularis* is *C. sp1* in Price et al. 2014).

Turtle ants typically adopt a low, flat body posture when foraging, with the legs oriented to the sides of the body (fig. 1C; de Andrade and Baroni Urbani 1999). *Cephalotes umbraculatus* is the only other species known to have workers that hold their gasters overhead when foraging. However, *C. umbraculatus* diverged from the clade containing *C. specularis* around 30 million years ago (Price et al. 2014) and the gaster-raising behavior of *C. umbraculatus* is coupled with independent foraging, conspicuous yellow patterning on the gaster, and the release of pungent defense chemicals (Coyle 1966). Distasteful defense chemicals and apparent aposematic coloration appear to be common in the turtle ants (Coyle 1966; de Andrade and Baroni Urbani 1999). It is therefore particularly noteworthy that *C. specularis* workers have uniform dark coloration and raise their gasters when interacting with a heterogeneric species that has the same coloration and posture. It suggests the co-option of the behavior for enhancing visual mimicry from the viewing perspective of the host, instead of for broadly advertising chemical defenses.



**Figure 1:** A, The stereotyped, raised body posture of a foraging *Crematogaster ampla* worker; B, the same raised body posture seen in a foraging worker of the turtle ant *Cephalotes specularis* when running in *C. ampla* foraging trails; and C, the typical flat body posture of a foraging turtle ant worker, illustrated here by *Cephalotes maculatus* that is commonly found outside of *C. ampla* territories at the study site. Photos by Scott Powell.

While running in *C. ampla* foraging trails, *C. specularis* workers avoid direct contact. This includes skirting the edge of trails with particularly dense traffic that leaves little room for contact-free running in a more internal position. Host workers do a “double-take” when they contact a passing *C. specularis* worker within the foraging traffic: the parasite worker elicits a brief alarmed response but moves on before the host workers can fully react. At food sources, like our baits, workers of the two species feed side by side (fig. 2B). Nevertheless, *C. specularis* workers actively avoid contact when approached, and *C. ampla* workers become alerted when they do manage to touch a *C. specularis* individual and then chase them (video 1, available online).



**Figure 2:** A, Two *Cephalotes specularis* workers feeding side by side on baited bark, with their shiny gasters reflecting the sky and canopy vegetation patterns overhead. B, *Crematogaster ampla* (left) and *C. specularis* (right) workers feeding side by side on a baited leaf. C, Foraging *C. ampla* workers next to a *C. specularis* nest entrance blocked by the armored dorsal surface of a soldier's head. Photos by Scott Powell.

This behavior suggests that the putative profile-view visual mimicry by *C. specularis* workers may dupe *C. ampla* only when there is no direct contact. It also indicates that *C. specularis* workers do not chemically mimic *C. ampla* to a degree that is sufficient to avoid detection when touched, as is universally true for within-nest social parasites (Lenoir et al. 2001; Buschinger 2009).

Some degree of chemical mimicry would complement and potentially enhance the effectiveness of the unique visual mimicry we describe here. Nevertheless, the aggressive response of *C. ampla* to *C. specularis* workers following direct contact indicates that chemical mimicry is incomplete at best in this system. This is to be expected, based on how complete chemical mimicry is typically

achieved by ant social parasites. Ant parasites can gain initial access to the host colony by having a neutral chemical signature, a base chemical profile that closely mimics that of their host ant species, or by releasing propaganda pheromones (reviewed in Lenoir et al. 2001; Buschinger 2009). In the cases of close mimicry, the similarity can be achieved by close descent or convergent evolution (e.g., Lenoir et al. 2001; d'Ettoire et al. 2002; Brandt et al. 2005b). Nevertheless, for all access strategies, it is only after entering the nest that the parasite's cuticular hydrocarbons, used in nestmate recognition in ants, can fully match those of the host colony (reviewed in Lenoir et al. 2001; Buschinger 2009). Indeed, a significant component of a colony's hydrocarbon profile is environmental and can only be acquired by shared food and constant contact (Liang and Silverman 2000; Lenoir et al. 2001). The nature of the interaction between *C. specularis* and *C. ampla*, exclusive to the foraging arena, would therefore appear to preclude the close contact necessary for fully acquiring host colony odor. Indeed, the lack of opportunity for complete chemical mimicry may explain the unique visual mimicry in this system: no other ant social parasitism occurs outside the nest, which significantly shifts the selection regime from intimate social contact in the dark to constant visual cues in an open, high-light environment. The possibility remains that the chemical profile of *C. specularis* might be more neutral or closely matching that



**Video 1:** Still photograph from a video (video 1, available online) showing a worker of the social parasite *Cephalotes specularis* avoiding contact with host *Crematogaster ampla* workers at a liquid food source applied to a tree trunk within the host colony's territory. The *C. specularis* worker is identified with an arrow at the start of the video, and the footage repeats a second time with two avoidance maneuvers slowed to 25% normal speed. Note the accelerated speed and looping path of the avoidance maneuvers and the alarmed response of the group of *C. ampla* workers that are contacted in the second maneuver. Footage by Scott Powell.



of their host than other turtle ants. This would complement the visual mimicry with a degree of chemical invisibility to their host that is only broken with direct contact. The detailed comparative tests needed to address this issue robustly are beyond the scope of this study but would be a valuable topic for further investigation.

All considered, our evidence suggests that *C. specularis* is a visual Batesian-Wallacian mimic of *C. ampla*. Batesian-Wallacian mimicry is defined as a type of aggressive mimicry where the ant model is also the dupe, and the mimic establishes a parasite-host relationship with its model (Pasteur 1982). This class of mimicry is common in solitary arthropod taxa (McIver and Stonedahl 1993), but to our knowledge, we have identified the first example of ant-ant visual Batesian-Wallacian mimicry. The benefit to *C. specularis* is the ability to infiltrate the heavily defended foraging network of the host species, to kleptoparasitize food resources. Ant-ant visual mimicry in the foraging arena has been identified before but only as apparent examples of Batesian mimicry, where the model is chemically defended and has conspicuous warning coloration (e.g., Ito et al. 2004).

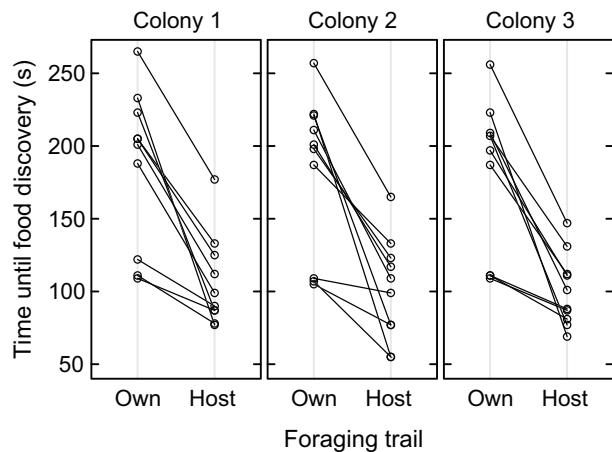
Batesian mimicry, especially driven by bird predation, is worth considering as an alternative to any form of visual mimicry where the ant model is proposed as the dupe. However, bird predation was never seen on the *C. ampla* trails in hundreds of hours of field time. In contrast, the model *C. ampla* is hyperaggressive to other ants, chasing and often killing any territorial intruders. The model therefore represents an overwhelmingly more significant source of mortality to heterospecific ants than any other known organism. Moreover, the posture mimicry of *C. specularis* is most apparent from the profile perspective of *C. ampla* and is likely to be readily visible to the host ants. Arboreal ants typically have large absolute and relative eye size (e.g., de Andrade and Baroni Urbani 1999; Ward and Downie 2005; Azorsa and Sosa-Calvo 2008) and visual systems that are critical for orientation and landmark recognition (e.g., Carroll and Janzen 1973; Baader 1996; Jander and Jander 1998; Ehmer 1999; Yanoviak and Dudley 2006), prey capture (e.g., Wilson 1962; Dejean et al. 2012), and enemy avoidance (e.g., Coyle 1966) in the high-light canopy environment. This explains the high incidents of visual Batesian-Wallacian mimicry of arboreal ant taxa by a wide diversity of solitary arthropod taxa (McIver and Stonedahl 1993). Critically, the accessibility of this interaction will allow future tests of how disruption of the *C. specularis* visual mimicry (e.g., preventing gaster raising, breaking the color matching with colored paint) impacts capture rates by the proposed dupe of *C. ampla*, versus other possible enemies that have yet to be identified.

#### Parasite-Host Co-Occurrence, Nesting Ecology, and Colony Size

The random transect-based survey of turtle ant diversity and abundance located a total of 285 colonies and 17 species. Only five *C. specularis* colonies were located in this survey, constituting 2% of all turtle ant colonies within the focal community. All *C. specularis* colonies nested within the territory of a *C. ampla* colony. The subsequent survey to assess the ecological co-occurrence rate between the two species found that 34 of 38 *C. ampla* colonies (89%) hosted *C. specularis* colonies within their territories. All baits were dominated by hundreds of *C. ampla* workers, and no more than four *C. specularis* workers were seen simultaneously at any one bait that they attended (71 of 192 baits). Two *C. ampla* colonies had two *C. specularis* colonies with nonoverlapping foraging ranges within their territory. Consequently, a total of 36 *C. specularis* colonies were located across the 38 host colonies. This high abundance of *C. specularis* colonies was in stark contrast to their 2% relative abundance in the random survey (contrasting *C. specularis* discovery per baited focal tree;  $\chi^2$  test, simulated *P* value with 2000 replicates,  $\chi^2 = 1,666$ ,  $P < .0005$ ). These findings demonstrate that within the focal community, *C. specularis* appears to be found exclusively within *C. ampla* territories, consistent with an obligate parasite-host association for the turtle ants.

For the three collected *C. specularis* colonies, there was no aggression among putative nestmates and clear aggression among putative nonnestmates, supporting the robustness of our colony collection method. Colony sizes (workers plus soldiers) for the colonies were 235, 288, and 676 individuals, respectively, and all had a single queen. Both the smallest and largest colonies had reproductive brood, demonstrating reproductive maturity at a colony size of approximately 200 individuals. Colonies occupied 6, 8, and 11 nests, respectively, consistent with the multiple-nest nesting ecology (polydomy, following Debout et al. 2007) recorded for other turtle ants (Powell 2009; Powell and Dornhaus 2013; S. Powell, unpublished data). Concordant with the intimately connected foraging ecology of the two species, all *C. specularis* nests were on trees numerically dominated by *C. ampla*, and therefore next to active host foraging trails (fig. 2C). Colony size estimates for the huge *C. ampla* colonies were not attempted. However, territorial arboreal *Crematogaster* species have been estimated to contain at least tens of thousands of worker (Tschinkel 2002). Reproductively mature *C. specularis* colonies therefore live within the territories of host colonies that are at least 2 orders of magnitude larger.

The exclusive association of *C. specularis* colonies with *C. ampla*, the high relative frequency of co-occurrence, and substantial differences in colony size meet a number



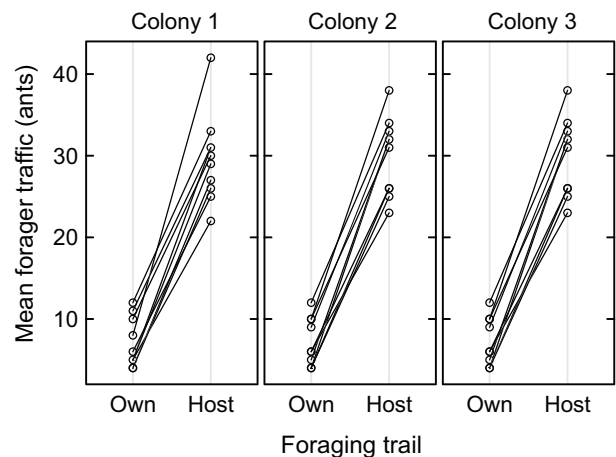
**Figure 3:** Dotplots for three *Cephalotes specularis* colonies, showing the time until food discovery along paired paths leading to food. Lines connect the paired data points from each trial. In each paired trial, one path had been used previously by the focal *C. specularis* colony and the other had a pheromone trail of *Crematogaster ampla*.

of key patterns expected for a mimetic social parasite. First, the exclusive association of 41 *C. specularis* colonies with *C. ampla* across two sampling approaches strongly suggests an obligate social parasitism. Consistent with the definition of xenobiotic social parasitism, the parasite nests and cares for its brood independently, and parasitizes a resource of its host (Buschinger 2009). However, a novel aspect of this xenobiotic interaction is that the multiple nests of each parasite colony are dispersed across the host's territory, allowing wide access to the trails needed to acquire the host's resources. Second, the near-complete saturation of host colonies is exceptional for a social parasite (Buschinger 2009), and may be indicative of a limited coevolutionary response for extirpating parasite colonies. It is worth noting that *C. specularis* is a young species (*C. specularis* is *C. sp1* in Price et al. 2014), so the time for coevolutionary responses by *C. ampla* may have been limited. Third, the small colony size of *C. specularis* meets the prediction that a mimic should coexist at a significantly lower relative frequency than their model, to minimize discovery by the dupe (Pasteur 1982). The substantially smaller colony size of *C. specularis* ensures that their workers are at very low relative frequency in the foraging trails and at food sources of their model and dupe, *C. ampla*. Indeed, the alarmed response of host workers to direct contact with the parasite, combined with the parasite's rapid avoidance behaviors (video 1), suggest that higher frequencies would be increasingly more dangerous for the parasite. This could be tested in future work by manipulating parasite-host relative frequencies in foraging traffic and measuring detection rates. Generally, we can view *C.*

*specularis* soldiers as a preadaptation for successful nesting within *C. ampla* territories (fig. 2C), while the mimetic workers are "ghosts in the system" that no one host individual has the opportunity to interact with for long. This contrasts strongly with the long-lasting and frequent interactions typical of within-nest xenobiotic social parasites and brood social parasites (Buschinger 2009).

#### *Eavesdropping and Foraging Efficiency Experiment*

Experiments in the laboratory were used to test a key hypothesis derived from our characterization of the parasite-host interaction: that the parasite's foraging performance is enhanced by the capacity to eavesdrop on the host's pheromone-based foraging trails. *Cephalotes specularis* colonies discovered food significantly faster along paths with *C. ampla* trail pheromones than along both control paths (paired *t*-tests,  $P \leq .001$  for all 3 colonies) and, remarkably, paths used previously by their own foragers (paired *t*-tests,  $P \leq .0005$  for all 3 colonies; fig. 3; data underlying analyses and fig. 3 are deposited in the Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.kj928> [Powell et al. 2014]). Similarly, sustained foraging activity, captured by mean forager traffic during a trial, was significantly higher along *C. ampla* pheromone trails than both the control path (paired *t*-tests,  $P \leq .0009$  for all 3 colonies) and each *C. specularis* colony's own trail (paired *t*-tests,  $P < .0001$  for all 3 colonies; fig. 4; data underlying analyses and fig. 4 are deposited in the Dryad Digital Repository <http://dx.doi.org/10.5061/dryad.kj928> [Powell et al. 2014]). In all trials, *C. specularis* foragers



**Figure 4:** Dotplots for three *Cephalotes specularis* colonies, showing the mean forager traffic along paired paths leading to food. Lines connect the paired data points from each trial. In each paired trial, one path had been used previously by the focal *C. specularis* colony and the other had a pheromone trail of *Crematogaster ampla*.

adopted the stereotyped stilt-leg and raised-gaster posture seen in the field (fig. 1B). This demonstrated that the presence of *C. ampla* foragers or trail pheromone is not necessary to elicit the posture mimicry in *C. specularis*, and it may therefore be a hard-wired foraging behavior.

Heterospecific trail following, a form of interceptive eavesdropping (Haynes and Yeargan 1999; Peake 2005), has been identified in a small number of other ants (e.g., Menzel et al. 2010), including in another turtle ant species (Adams 1990). However, the “faster and stronger” foraging performance of *C. specularis* along the pheromone tails of a heterogeneric species compared to their own trails is unprecedented. Moreover, previous examples of heterospecific trail following appear to be facultative, and the dominant species does not tolerate heterospecific exploitation of rich food resources (Adams 1990; Menzel et al. 2010). It is this almost universal intolerance by ant colonies of conspecific and heterospecific foragers at rich food sources that likely explains the rarity of the evolution of heterospecific pheromone-trail following. Nevertheless, all current evidence suggests that *C. specularis* follows *C. ampla* trails obligately, or that it is at least the standard foraging mode for *C. specularis* in normal ecological contexts. This is even more remarkable given the exceptional intolerance of *C. ampla* to all intruders in trails and at food resources, including *C. specularis* workers when they are contacted (video 1). The strong visual mimicry and avoidance behaviors of *C. specularis*, allowing them to infiltrate the trails of their host and avoid detection and capture, may then explain their exceptional capacity for heterospecific trail following: once largely invisible to the trail-owning species, constraints on selection for improved heterospecific trail following may have been lifted. It will therefore be valuable to contrast any capacity for heterospecific trail following in the close relatives of *C. specularis*, to determine whether their exceptional trail-following ability is coincident with or precedes the evolution of the other components of the social parasitism.

Interceptive eavesdropping has been widely demonstrated to have negative consequences when the intercepted signal is part of an intraspecific communication system (Haynes and Yeargan 1999; Peake 2005). The rarity of heterospecific trail following in ants, and the general intolerance of trail owners toward intruders, is therefore not surprising. Nevertheless, it is exactly these kinds of interactions that have offered invaluable insights into the coevolutionary arms race between the exploited and exploiting species (e.g., Zuk and Kolluru 1998; Haynes and Yeargan 1999; Peake 2005; Gonçalves et al. 2008; Tizo-Pedroso and Del-Claro 2014). In this case, the special nature of the interaction between *C. specularis* and *C. ampla* offers a unique opportunity to understand the coevolu-

tionary arms race between signaler and eavesdropper, and host society and social parasite more generally.

### Conclusions

Here we have demonstrated that the newly discovered mirror turtle ant, *C. specularis*, visually mimics a heterogeneric ant host, lives in exclusive ecological association with the host, and efficiently eavesdrops on the host's network of pheromone-based foraging trails to exploit guarded and otherwise inaccessible food resources. Considered together, these characteristics constitute a novel adaptive syndrome of social parasitism. In established terminology, it combines the first apparent case of Batesian-Wallacian mimicry in ants, exceptional pheromone-based interceptive eavesdropping, kleptoparasitism of guarded food resources, and obligate xenobiotic nesting ecology. Critically, this novel species interaction appears to represent an early stage in the evolutionary transition from a free-living species to a highly derived and functionally reduced social parasite. The ready access to this interaction in the low savanna canopy, and the relative ease with which colonies can be collected and manipulated, makes this system particularly amenable to experimental studies. In this work, we used the experimental tractability of the system to identify the exceptional eavesdropping capabilities and foraging performance of the parasite on the host's pheromone trails. Future experimental studies, examples of which we have outlined here, will maximize the insights gained from this system, as will comparative analyses of trait evolution in *C. specularis* and its close relatives. Broadly, this newly identified and characterized interaction offers a special opportunity to better understand the ecological drivers of social parasitism, and the components of the novel adaptive syndrome that it represents.

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A worker of the mirror turtle ant, *Cephalotes specularis*, returning home to a nest entrance guarded by the armored head of a soldier. While the unique mimicry of the workers allows them to operate undetected in the host colony's foraging network, soldiers are critical for defending the parasite's nesting cavities within the host's territory. Photo by Scott Powell.