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Dead ant walking: a myrmecophilous beetle predator uses parasitoid host location cues to selectively prey on parasitized ants

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Myrmecophiles (i.e. organisms that associate with ants) use a variety of ecological niches and employ different strategies to survive encounters with ants. Because ants are typically excellent defenders, myrmecophiles may choose moments of weakness to take advantage of their ant associates. This hypothesis was studied in the rove beetle, Myrmedonota xipe, which associates with Azteca sericeasur ants in the presence of parasitoid flies. A combination of laboratory and field experiments show that M. xipe beetles selectively locate and prey upon parasitized ants. These parasitized ants are less aggressive towards beetles than healthy ants, allowing beetles to eat the parasitized ants alive without interruption. Moreover, behavioural assays and chemical analysis reveal that M. xipe are attracted to the ant’s alarm pheromone, the same secretion used by the phorid fly parasitoids in host location. This strategy allows beetles access to an abundant but otherwise inaccessible resource, as A. sericeasur ants are typically highly aggressive. These results are the first, to our knowledge, to demonstrate a predator sharing cues with a parasitoid to gain access to an otherwise unavailable prey item. Furthermore, this work highlights the importance of studying ant–myrmecophile interactions beyond just their pairwise context.

1. Introduction

Ant societies attract a suite of symbiotic organisms that take advantage of a colony’s abundant resources. Beetles in the family Staphylinidae are common ant associates, yet relatively little is known about the role of these beetles in their host colonies. Owing to the formidable chemical and behavioural defences of ants, beetles often possess complex strategies to safely interact with their ant symbionts [1,2]. Many beetles act as scavengers that hide in refuse piles, or as ant-mimicking social parasites within a host colony, draining them of resources. Others are predators of the ants themselves, locating their prey by ‘eavesdropping’ on the ants’ communication system [3]. While commensal, parasitic and predatory beetles are common within ant societies, little is known about how beetles might benefit their ant associates [4]. Furthermore, although most biological communities function as complex networks of context-dependent interactions, ant–beetle associations are rarely observed outside of their pairwise context.

While often studied in isolation, predator–prey and host–parasitoid interactions have a wide array of effects within food webs and are thus increasingly approached from a community perspective [5]. Natural enemies can directly influence hosts or prey by reducing population size or inducing changes in phenotype (e.g. behaviour, morphology). These interactions often have cascading effects on other species within the community [6–8]. Cascading (or indirect) effects can be mediated by both changes in host/prey density (density-mediated indirect effects) and changes in the traits of host/prey species (trait-mediated indirect effects) (reviewed in [9]).
Trait-mediated interactions may be particularly relevant to host–parasitoid systems because host behaviour and/or physiology is frequently modified as a result of parasitism, with subsequent consequences for structuring biological communities [10]. Trait modifications can occur before or after parasitism and are either adaptive or non-adaptive to the parasitoid and/or host [11–13]. For example, in the presence of parasitoids, hosts will often suspend normal activity in order to implement chemical or behavioural defensive strategies [14–16]. After parasitism, immature parasitoids within the host may alter the host’s physiology to encourage behaviours that optimize conditions for parasitoid development [17–19]. Alternatively, host immune response or even self-sacrifice behaviour may increase to prevent parasitoids from developing [20,21].

Here, we experimentally demonstrate a context-dependent predation strategy involving: (i) phorid fly parasitoids, where parasitism reduces ant aggression; and (ii) predatory beetles, where beetles selectively prey upon parasitized ants, thus potentially reducing phorid fly populations without impacting ant populations. We also demonstrate that this interaction is mediated by the alarm pheromone of the ant, which both natural enemies use in host/prey location.

Azteca sericeasur (referred to as Azteca instabilis in prior publications, but recently identified as A. sericeasur; J. Longino 2014, personal communication) is a highly aggressive and territorial arboreal ant species that lives in large polydomous carton nests [8]. This species frequently nests in the shade trees of coffee plantations and forages in the coffee below, preying on and removing herbivores [22]. Ants in the genus Azteca are known for their pungent alarm pheromone that they disperse liberally from large pygidial gland sacs when disturbed. A suite of Pseudacteon phorid fly species uses this alarm pheromone to locate and parasitize A. sericeasur [23]. Pseudacteon lascinosus is the largest of the three species and the most abundant at our field sites and Pseudacteon planidorsalis is a smaller species and the second most abundant [24]. The presence of phorid fly parasitoids not only reduces the ants’ ability to forage by as much as 50% [16,23], but also indirectly affects interactions between the ants and a wide range of competitors and mutualistic partners [8]. The newly described species of rove beetle, Myrmelonota xipe (Staphylinae), has been observed in association with A. sericeasur ants. Myrmelonota xipe beetles are found near disturbed A. sericeasur, often mating or preying on A. sericeasur ants after the arrival of phorid flies [26]. These interactions suggest two questions. First, because A. sericeasur workers are notoriously aggressive, how are the beetles able to prey upon the ants? Second, are the beetles using the same alarm pheromone as the phorid flies to locate the ants?

We test the hypothesis that parasitism by phorid flies reduces ant aggression, allowing beetles to gain access to the ants as a prey item. Furthermore, we hypothesize that this context-dependent interaction is induced by the ant’s alarm pheromone, which is released during phorid attack.

2. Material and methods

(a) Study site

We conducted all fieldwork on a shaded coffee plantation, Finca Irlanda, in the Soconusco region of Chiapas, Mexico (15°11’N, 92°20’W) between July 2012 and March 2013. Finca Irlanda is approximately 280 ha in size, located between 950 and 1150 m elevation, and receives approximately 4500 mm of precipitation per year. A. enephera is the most abundant ant of the approximately 60 species of arboreal ants on the farm [25]. A. sericeasur builds carton nests on the trunks of shade trees within the coffee plantation, where their colonies tend to be distributed in patches [27]. Ants, beetles and phorid flies were all collected for laboratory experiments at five different A. sericeasur nests within the field site using an aspirator.

(b) Arena experiment

To determine whether M. xipe selectively attack parasitized ants, and whether parasitized and unparasitized ants respond to beetle attacks differently, we placed parasitized and unparasitized ants in an arena with the beetles, filmed their interactions, and analysed the resulting footage (electronic supplementary material, S1). We placed A. sericeasur ants (n = 5) in small plastic container with an individual phorid fly until the ants were parasitized (approx. 1 h). We then chilled the containers in the −20°C freezer for 2 min until the fly and ants were anaesthetized. While the ants were anaesthetized, we confirmed that ants were successfully parasitized by inspecting for oviposition wounds from the phorid fly. If the ant was successfully parasitized, we added a single dot of paint (green, white or blue chosen at random for each observation) on the head of each ant and identified the anaesthetized phorid flies to species under a microscope (electronic supplementary material, S2). New phorid flies were used for each parasitism event. We also anaesthetized and painted five unparasitized ants using the same method. We confirmed the ants to be unparasitized by inspecting them for a lack of oviposition wounds. We allowed the ants a 1 h recovery period, which was enough time for ants to resume normal activities. Parasitized ants were used within 4 h of the initial parasitism event. We placed the five unparasitized ants and five parasitized ants (by either P. lascinosus or P. planidorsalis) with two M. xipe beetles in a plastic container (arena) coated with fluxon (Northern Products, Rhode Island, USA) to prevent the insects from climbing the walls of the arena. We sealed the arena with a transparent glass lid. We then filmed the arena for 15 min. A total of 82 arena experiments were conducted (50 P. lascinosus- parasitized versus healthy and 32 P. planidorsalis- parasitized versus healthy treatments). We later analysed video footage of the arena experiments using OBSERVER XT software (v. 11, Noldus Information Technology, Wageningen, The Netherlands), by recording the duration of each behaviour. The behaviours we included were: (i) attack (one animal bites another animal); and (ii) mandible flare (an ant opens and closes her mandibles repeatedly in the direction of another animal) [28]. We recorded behaviours with the OBSERVER Software, blind to the treatment type. We calculated the total duration of each behaviour by organism type (parasitized ant, unparasitized ant or M. xipe) and the corresponding target organism type for each observation.

(c) Beetle trap experiment

Previous studies have shown that dying ants will leave their nests or are restricted entry to their nests [29]. To determine whether M. xipe beetles are able to selectively locate individual parasitized ants within the coffee plantation, we constructed beetle traps from small plastic cups with plaster of Paris on the bottom to retain moisture and lids with holes large enough for the beetles to enter the traps (electronic supplementary material, S3). Each cup contained an A. sericeasur worker randomly assigned to one of four treatment types. The four treatments included: (i) A. sericeasur parasitized by P. lascinosus, (ii) A. sericeasur parasitized by P. planidorsalis, (iii) A. sericeasur manually injured by puncturing their mesothorax with a Minuten pin (0.20 mm diameter, Bioquip Products, Rancho Dominguez, CA, USA) to simulate a phorid attack wound, and (iv) A. sericeasur untreated as a control. Ants in parasitoid treatments were parasitized according to the same
methods in the arena experiment. We placed a total of 624 traps, divided evenly by treatment. We chose 39 sites in both high shade (10 sites with focal trees containing A. sericeasur nests and 10 sites without A. sericeasur nests) and low shade (10 sites with focal trees containing A. sericeasur nests and nine sites without A. sericeasur nests). We placed 16 traps at each site: one cup of each treatment type 0 m and 5 m from each sites’ focal tree, both on the ground and suspended 1 m in the nearest coffee plant. We placed the cups for each site in the field on the same day, and retrieved them 2 days later (electronic supplementary material, S4). We identified M. xipe beetles in each cup, and recorded whether the ant was living or completely consumed.

(d) Extraction and analysis of Azteca alarm pheromone
To determine the primary components of A. sericeasur alarm pheromone, we collected A. sericeasur volatile alarm pheromone compounds by first placing an A. sericeasur worker in a 50 ml glass beaker. Then we disturbed the worker by pinching her tibia and covered the beaker with aluminium foil. We then inserted a solid phase microextraction (SPME) fibre into the beaker through the aluminium foil for 10 min to adsorb the headspace volatiles. We conducted five ant volatile collections. SPME fibres were immediately inserted into a Finnigan Trace MS gas chromatograph/mass spectrometer equipped with a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, CA, USA). Extracts were analysed in splitless mode, with a temperature programme that started at 100°C for 1 min, which then increased by 20°C min⁻¹ until it reached 150°C, and then increased by 5°C min⁻¹ until it reached 325°C where it stayed for 5 min. Injector and transfer line temperatures were kept at 325°C and 280°C, respectively.

(e) Alarm pheromone bioassays
To determine whether M. xipe are attracted by the alarm pheromone of A. sericeasur, we obtained commercially available synthetic 2-heptanone and 2-pentanone (Sigma-Aldrich, St Louis, USA), the two primary compounds released by disturbed ants. We prepared four treatment solutions: (i) 1 ml of pesticide-grade hexanes as a negative solvent control, (ii) 20 crushed A. sericeasur pygidial glands in 1 ml of hexane, (iii) 5 μl 2-heptanone in 1 ml of hexane, and (iv) 5 μl of 2-pentanone in 1 ml of hexane. We then placed treatment solutions in 2 dram glass vials, open, with a filter paper wink at 20 field sites [23]. All field sites were at least 25 m apart, at the base of trees within the coffee farm that contain an A. sericeasur nest. At each site, we placed the treatment solution vial on the ground with leaf litter removed from the surrounding area. Once we opened the vial with treatment solution, we observed a 10 cm² area surrounding the vial for 15 min, and collected and identified beetles attracted to the area using an aspirator. We quantified the total number of beetles from each species collected at each site with each treatment type.

(f) Data analysis
All statistical analyses were performed in R (v. 3.1.2) [30]. We tested for significant differences in prey choice (parasitized or healthy control), sites (1–39), distance from focal tree (0 m or 5 m), the presence or absence of an A. sericeasur nest at focal tree, trap height placement (0 m or 1 m) and shade cover (high or low) as fixed effects. In the second model, we added interactions between treatment and height, and between treatment and shade. In the third model, we added all interactions between treatment and each habitat variable. To select the best model, we used the Akaike’s information criterion (AIC) provided by the ‘mass’ package and after we fitted the models we checked homoscedasticity of the residuals [31]. The model that best predicted ant removal by beetle predators was the first model, thus including interactions between habitat variable and treatments did not improve fit of the model. For the alarm pheromone bioassays, we tested for significant differences between treatments (2-heptanone, 2-pentanone, pygidial gland extract positive control or hexane negative control) using a one-way ANOVA followed by Tukey HSD mean comparison tests.

3. Results

(a) Do beetles prefer to prey on parasitized ants?
In the arena experiment, beetles attacked ants that were parasitized by P. lasciniosus more often than they attacked unparasitized ants (figure 1a; ANOVA; F1,48 = 24.59, p < 0.001). Ants parasitized by P. planidorsalis were not attacked more than parasitized ants (figure 1b; % of observation spent attacking: 11.21 ± 5.67% spent attacking parasitized ants, 8.97 ± 6.23% spent attacking unparasitized ants; ANOVA; F1,31 = 0.81, p = 0.37). In the beetle trap experiment, beetles were found consuming only ants parasitized by either P. lasciniosus (14.7% of ants) or P. planidorsalis (2.5%) and did not consume control or injured (sham-parasitized) ants. Consumption of P. lasciniosus-parasitized ants was significantly higher than consumption of control or injured ants (GLMM, χ² = 50.33, p < 0.0001; figure 2), and consumption of P. planidorsalis-parasitized ants was not significantly higher than control or injured ants (GLMM; χ² = 50.33, p = 0.20).

(b) Do parasitized and unparasitized ants display different levels of aggression towards predatory beetles?
In the arena experiment, parasitized ants were less aggressive than healthy ants. During the observations, parasitized ants attacked beetles significantly less often than did unparasitized ants, regardless of which phorid species parasitized them (figure 1c,d; P. lasciniosus-parasitized ants × unparasitized ants: F1,34 = 4.124, p < 0.05; P. planidorsalis-parasitized ants × unparasitized ants: F1,26 = 4.495, p < 0.05). However, ants parasitized by the two species of phorid fly differed in their mandible flare performance when compared with unparasitized ants. Ants parasitized by P. lasciniosus flared their mandibles less often than did unparasitized ants (figure 1e; F1,40 = 11.38, p < 0.002), but ants parasitized by P. planidorsalis did not (figure 1f; F1,28 = 0, p = 0.99).

(c) Are beetles able to successfully locate parasitized ants in different habitat types?
In the beetle trap experiment, ants were completely consumed in all traps where M. xipe were collected after 2 days. Beetles
Figure 1. Box plots of arena experiment behaviours. (a) Beetles attacking ants parasitized by *P. lasciniosus* (left column) and healthy ants (right column). (b) Beetles attacking ants parasitized by *P. planidorsalis* (left column) and healthy ants (right column). (c) *Pseudacteon lasciniosus*-parasitized ants (left column) and healthy ants (right column) attacking beetles. (d) *Pseudacteon planidorsalis*-parasitized ants (left column) and healthy ants (right column) attacking beetles. (e) Mandible flares by *P. lasciniosus*-parasitized ants (left column) and healthy ants (right column). (f) Mandible flares by *P. planidorsalis*-parasitized ants (left column) and healthy ants (right column). The box plots indicate the median (large horizontal bars), the 25th and 75th percentiles (white rectangles), the minimum and maximum values (whiskers) and outliers (white circles). Asterisks represent significance level (* p < 0.05, ** p < 0.01, *** p < 0.001). (Online version in colour.)

Figure 2. Proportion of ants consumed by *M. xipe* beetles in beetle trap experiments. Bars represent the four treatment types: controls are traps baited with healthy ants, injure treatments are traps baited with manually injured ants, *P. planidorsalis* treatments are traps baited with ants parasitized by *P. planidorsalis* and *P. lasciniosus* treatments are traps baited with ants parasitized by *P. lasciniosus*. Asterisks indicate significance level (*** p < 0.001).

Successfully located and consumed ants parasitized by *P. lasciniosus* in both habitat types. Attacks on ants in high shade were significantly more frequent than attacks in low shade (GLMM; figure 3a; *P. lasciniosus* × shade, $\chi^2 = 15.41$, $p < 0.0001$) and significantly more frequent on the ground than above ground (GLMM; figure 3b; $\chi^2 = 9.21$, $p < 0.003$). Furthermore, there were no differences based on distance from focal tree (GLMM; figure 3d; $\chi^2 = 2.65$, $p = 0.10$), or with and without *A. sericeasur* presence (GLMM; figure 3c; $\chi^2 = 0.06$, $p = 0.80$).

4. Discussion

Our results show that *M. xipe* selectively preys on parasitized ants, particularly those parasitized by *P. lasciniosus*. These parasitized ants display reduced aggression (including a reduced frequency of mandible flaring), which may allow beetles to more easily gain access to them as a prey item. Furthermore, in the field, beetles were able to consume up to 14.7% of ants parasitized by *P. lasciniosus*, which suggests that these beetles may have an important role in reducing *P. lasciniosus* populations. This preliminary estimate of beetle predation rates should be tested in further studies. However, it is probably conservative, given that *M. xipe* are generally already present just after phorid parasitism and are frequently observed preying on ants near disturbed colonies shortly after parasitism takes place (KA Mathis 2014, personal observation). *Myrmedonota*...
xipe preyed on ants parasitized by *P. planidorsalis* significantly less often than it preyed ants parasitized by *P. lasciniosus*, probably because ants parasitized by *P. planidorsalis* were more aggressive than ants parasitized by *P. lasciniosus*. These experiments indicate that beetles probably have a stronger impact on ant–*P. lasciniosus* interactions than ant–*P. planidorsalis* interactions.

This study also demonstrates that *M. xipe* uses *A. sericeasur* alarm pheromone as a cue to locate prey. Although phorid flies also use the alarm pheromone to locate *A. sericeasur* hosts, they are attracted to 1-acetyl-2-methylcyclopentane, a less abundant compound within the alarm pheromone blend that is only found in *Azteca* species ants [23,32,33]. The compound that attracts *M. xipe*, 2-heptanone, is relatively common in the alarm pheromone of dolichoderine ants. The use of 2-heptanone by *M. xipe* suggests that these beetles may be less selective and may prey on other dolichoderine ant species in addition to *A. sericeasur*. However, of the approximately 15 species at our study sites, *A. sericeasur* is overwhelmingly the most abundant, and are probably the ant that these beetles encounter most frequently.

Several other studies have shown intraguild predation of parasitoids, including examples where: (i) a predator preys on the adult parasitoid as well as the host, (ii) a predator preys on the unparasitized hosts as well as hosts with an ectoparasitoid, (iii) a predator preys more on parasitized hosts than healthy hosts owing to host vulnerability after parasitism, and (iv) a predator preys more on unparasitized hosts owing to the more advantageous location of parasitized hosts in a nest (reviewed in [34]). These studies differ from ours in that *M. xipe* appears to almost exclusively prey on parasitized ants, as they are unable to access unparasitized ants as a resource.

The unique strategy of *M. xipe* may have adaptive implications for both *A. sericeasur* and the phorid parasitoids. First, as beetles are only consuming individuals that harbour phorid fly eggs, beetle predation may, counter-intuitively,
benefit its prey by reducing the phorid fly population and thus predation may be adaptive to *A. sericeasur*. Adaptive suicide in social insects is relatively common as a pre-emptive strategy (e.g. honeybees using their stings to defend the colony), although it is notably difficult to evaluate in the context of host–parasitoid interactions [29,35–39]. The host suicide hypothesis postulates that mature parasitoids emerging from hosts are more likely to infect host’s kin than non-kin. Therefore, when maturation of the parasitoid is prevented, the inclusive fitness of the host should be increased. Even a very small increase in inclusive fitness will be enough to drive the system, and favourable situations for adaptive suicide include systems where the host is a social insect, when there is high host inbreeding, and when parasitoids have small search ranges [20]. This system appears to be a good candidate to meet these criteria, as *A. sericeasur* is not only a social insect, but is also polygynous and polydomous, forming colonies that can have territories spanning several hectares [40]. Although the lifetime dispersal distances of these phorid flies are currently unknown, *P. lascinosus* and *P. planidorsalis* are rarely found farther than two meters from any given *A. sericeasur* nest [41]. Therefore, it is unlikely that phorid flies are dispersing beyond the boundaries of a single *A. sericeasur* colony prior to oviposition, although future work on the precise dispersal distance of these phorid flies is needed.

However, despite the potential benefits, selective predation on parasitized ants may still have costs for the ant colony, and therefore, not support the host suicide hypothesis. First, it is possible that some small percentage of phorid fly larvae do not successfully mature to adulthood. In these cases, ants that survive parasitism and go on to benefit the colony would be at a greater risk for predation, which would ultimately cost the colony as a whole. Second, if the parasitized workers are active colony members during the phorid’s development period, the benefit of eliminating the phorid fly larvae may be offset by the costs incurred from losing productive parasitized ant workers. Future work is needed to confirm true costs and benefits of beetle predation on parasitized ants. Additionally, it will be worthwhile to determine the physiological mechanism by which this behavioural switch in aggression occurs in *A. sericeasur*.

Nonetheless, our study shows that the effects of the *M. xipe* association with *A. sericeasur* is dependent upon phorid fly presence and that these beetles may be indirectly beneficial to *A. sericeasur*, by reducing the number of developing *P. lascinosus* and *P. planidorsalis* parasitoids by approximately 14.7% and 2.6%, respectively. To our knowledge, ours is one of the few studies that document the role of ant-associated beetles outside of a pairwise context [2,42–46], and the first study to demonstrate a predator sharing host/prey cues with a parasitoid to gain access to a prey item that would otherwise be unavailable.

Increasingly, it is becoming apparent that investigating the ecological complexity within a system provides instructive examples of how organisms can change their behaviour or morphology in response to challenges from other organisms, and subsequently, how these changes can have cascading effects throughout a network of interacting species [8,9]. Current literature on the role of beetles within ant societies tells us that these beetles are exceedingly common and behaviourally diverse, but few ant–beetle associations have been examined in depth. Further investigation into both the roles of these beetles and their ant hosts, as well as within the network of organisms surrounding ant societies is crucial to understanding how social insect colonies function within the community as a whole.

Data accessibility. All data are available from the Dryad data repository: http://dx.doi.org/10.5061/dryad.g7599.

Authors’ contributions. K.A.M. conceived, designed and performed the experiments. K.A.M. and N.D.T. analysed the data and wrote the manuscript.

Competing interests. We declare we have no competing interests.

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