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Original Article

Do zombie ant fungi turn their hosts into light seekers?

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Specialized parasites can modify host behavior to benefit transmission and reproduction. Such behavior is considered an extended phenotype of the parasite. The interactions between certain ant species and fungi of the genus *Ophiocordyceps* form an evident example. Once infected by *Ophiocordyceps camponoti-atricipis*, *Camponotus atriceps* ants die, biting at specific sites where abiotic conditions are optimal for fungal development. For many species of free-living fungi, light is needed to induce growth of the reproductive stage. However, the role of light in *Ophiocordyceps* behavioral manipulation and fruiting body development is largely unknown. Here, we investigated the association between illuminance and the incidence of dead manipulated *C. atriceps* ants. We identified ant graveyards in the field and experimentally changed the incident illumination for half of each graveyard using shading screens. Such screens resulted in a clear reduction of incident light, as well as slightly higher, more stable humidity levels. We measured the appearance of recently died, infected ants, the height at which they were found, and their fruiting body production. The presence of dead infected *C. atriceps* was strongly influenced by experimental light reduction. Shaded areas harbored fewer recently infected ants compared to naturally illuminated areas. In addition, in shaded areas, a smaller number of ants produced fruiting bodies and these ants also appeared to have climbed to higher elevations in comparison to control areas. Our findings indicate that light influences the place of the *C. atriceps* death, and fungal development by seemingly affecting fruiting body formation in *O. camponoti-atricipis.*

Key words: **biting behavior, *Camponotus*, extended phenotype, incident light, *Ophiocordyceps*.**

INTRODUCTION

Specialized parasites can cause morphological, physiological, and behavioral modifications in their hosts that benefit parasite development, reproduction, and transmission (Combes 2001; Thomas et al. 2010). Benefits can be so extensive that parasites can be selected to promote dramatic changes in host behavior (Lefèvre et al. 2009). For instance, when host changes cause infective propagules to be released at sites that favor transmission, such behaviors are considered adaptive to the parasite (Biron et al. 2005). Manipulations of host behavior, which clearly enhance parasite gene propagation are, therefore, considered to be parasite-extended phenotypes (Dawkins 1982).

Ants infected by fungi of the *Ophiocordyceps unilateralis* species complex display a stereotypical biting behavior just before they die that does not occur in healthy ants (Hughes et al. 2011). This fungus-induced behavior appears to be adaptive to the temperate and tropical ecosystems from which manipulated, dead ants have been sampled (de Bekker, Ohm, Evans, et al. 2017; Araújo et al. 2018; Loreto et al. 2018). These so-called "zombie-ants" are an evident example of parasitic manipulation of host behavior. Infection likely occurs via contact between fungal spores and the cuticle of a foraging ant (Hughes et al. 2011). Subsequently, the fungus grows inside the ant's body where it is not infective to the ant's conspecifics and seems to go unnoticed by nest mates (Gracia et al. 2018). A few weeks after initial infection, ants start to show erratic and tremulous movements as they leave the nest to climb vegetation, often falling due to convulsions (Andersen et al. 2009; Hughes et al. 2011; de Bekker et al. 2015). Death follows when

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an infected ant attaches itself by its mandibles to a leaf or other plant part, such as twigs, spines, or stems (Andersen et al. 2009; Loreto et al. 2018). After successful manipulation, the fungus rapidly switches to consuming the ant (de Bekker et al. 2015) to produce an ascoma (i.e., fruiting body) that rises out of the pronotum of the ant. Here, infective spores are produced, which are effectively liberated into the environment, due to the elevated position of the sprouting cadaver (Evans and Samson 1984; Evans et al. 2011).

All known records of parasitism by fungi of the *Ophiocordyceps unilateralis* species complex come from members of the ant tribe Camponotini (Araújo et al. 2018). In fact, all current evidence suggests that ant-infecting *Ophiocordyceps* parasites have a specific relationship with their host. Each fungal species infects only a single species of ant (Loreto and Hughes 2016; Araújo et al. 2018; Sakolrak et al. 2018). Cadavers of infected ants can be encountered in large quantities (more than 26 individuals/m^2 for *Camponotus leonardi* in Thailand) at sites known as "graveyards" (Pontoppidan et al. 2009). Within such graveyards in evergreen primary forest of Thailand, the majority of infected ants are encountered dead at 25 cm above the forest floor (Andersen et al. 2009). At this height, temperature and humidity vary less than higher up in the forest vegetation and are thought to favor fungal spore development, release, and dispersal (Andersen et al. 2009). However, in the Atlantic Rainforests of Minas Gerais, Brazil, infected ants (*Camponotus rufipes*) died at higher elevations compared to Thailand, with a greater variance around the mean (60 to 180 cm). The reason for this phenotypic difference is unknown (Andersen and Hughes 2012).

The mechanisms that underlie parasitic behavioral manipulation still remain largely uncovered at this time. However, studies on caterpillars infected with baculovirus (Hoover et al. 2011) and crickets infected with nematomorphs (Thomas et al. 2002) recorded evident positive phototactic behaviors in infected hosts versus noninfected conspecifics (Ponton et al. 2011; Van Houte et al. 2013; Van Houte et al. 2015). It is well known that ants can use spatial references such as light and solar position as a means to orient themselves when foraging and returning to their nests (Wehner 1984; Wystrach and Graham 2012). Moreover, the presence of a light-entrainable biological clock has been demonstrated for ants (i.e., *Solenopsis invicta* and *Camponotus rufipes*) (Ingram et al. 2012; Mildner and Roces 2017), as well as for *Ophiocordyceps kimflemingiae* (a species within the *unilateralis* complex) (de Bekker, Will, et al. 2017). Yet, the use of light by parasitic fungi, as a means to position ant hosts under conditions that facilitate spore production and transmission, remains a novel topic. Several studies, albeit with different approaches, have suggested that light may play a role in establishing fungal manipulation of ant behavior (Hughes et al. 2011; de Bekker et al. 2014; de Bekker et al. 2015; Chung et al. 2017; de Bekker, Will, et al. 2017). However, the exact influence of sunlight on the behavioral modification of *Ophiocordyceps*-infected ants remains incipient. To begin elucidating the importance of light on fungal manipulation of ant behavior, we performed a field experiment in which incident illumination was experimentally manipulated in identified graveyards. As such, we investigated the influence of light intensity on position of death and fruiting body formation of a carpenter ant *Camponotus atriceps*, which is commonly found infected with *Ophiocordyceps camponoti-atricipis* in the Central Amazon of Brazil.

MATERIAL AND METHODS

Experimental field setup

Our study was undertaken in Ducke Forest Reserve, situated north of the Manaus city, Amazonas State, Central Amazonia, Brazil (02°55′ S, 59°59′ W). The reserve is a 100 km^2 area of tropical lowland rainforest that is not inundated by flooded rivers (i.e., *terra firme*, "firm earth" forest) (Alencar 1986). At this study site, 10 graveyards (i.e., sites where dead infected ants were found) were located. At each graveyard, a 10 × 10 m plot was delimited. Half of each plot was covered with an 80% polyethylene shading screen installed at 2 m off the forest floor (Figure 1). Shading screens were positioned such that the number of fungus-infected cadavers already present was similar in adjacent shaded and unshaded areas. Screens were then oriented in north–south direction such that solar incidence was uniform in both shaded (treatment) and unshaded (control) areas throughout the day. After adjusting shading screen position, moving it in a north–south direction, ants were recounted when necessary to assure that half of the already present ants was in each treatment. Subsequently, all fungus-infected ant cadavers, both already present and newly found, were marked with individually numbered colored tags. Statistical analyses only included the infected individuals that appeared after the installation of the shading screens. To account for the more intense entrance of lateral illumination during sunrise and sunset, a buffer zone of 50 cm in height and 50 cm in width was delineated at the borders of the shaded areas. To maintain comparability, a border area of the same proportions was marked in the unshaded control areas.

Monitoring for newly infected *C. atriceps* cadavers was conducted weekly between October 2016 and April 2017. The location (shaded, control or border), height above the ground, and the presence or absence of fungal ascomas of each newly encountered ant cadaver was measured (Figure 1a–c). Data collection occurred by ways of meticulous, weekly inspections in the 10 plots that included trees, palm-trees, shrubs, and the herbaceous understory.

Recording of abiotic factors

We used 2 sensor towers to collect data on relative humidity, air temperature, and light intensity. Towers were positioned centrally in the shaded and control areas to measure abiotic variations. Each tower contained a sensor that measured light intensity as incident solar radiation (μE) (Silicon Pyranometer Sensor model S-LIB-M003, connected to a Hobo Micro Station Data Logger, model H21-002, Onset), and a thermo hygrometer Data Logger (Icel brand, model HT-4000), which measured temperature (°C) and air relative humidity (%). The towers were set to high-density data collection and recorded data every hour. Each week, during each new round of ant cadaver monitoring, the towers were relocated to another study plot. As a result, data collection was equal for all plots for the duration of the experiment.

Species identification

Ant species were confirmed through morphological identification and comparison with specimens from INPA's Entomology Collection, followed by consultation with specialists. To identify the fungal parasite, 9 infected dead ants from 6 graveyards, with fresh fruiting bodies (ascomas), were collected in sterilized Eppendorf tubes for DNA extraction and genetics analysis. DNA extractions were performed on the day of collection using the DNeasy Plant Mini Kit extraction protocol (Qiagen), with a few modifications. Sections of each ascoma were inserted into 2 mL microtubes with 2 zircon beads and 200 μL AP1 buffer (Qiagen) for cell rupture using a Precellys® 24 macerator, for 1 min at 5000 rpm. Subsequently, an additional 200 μL of AP1 buffer and 4 μL of RNase A (provided

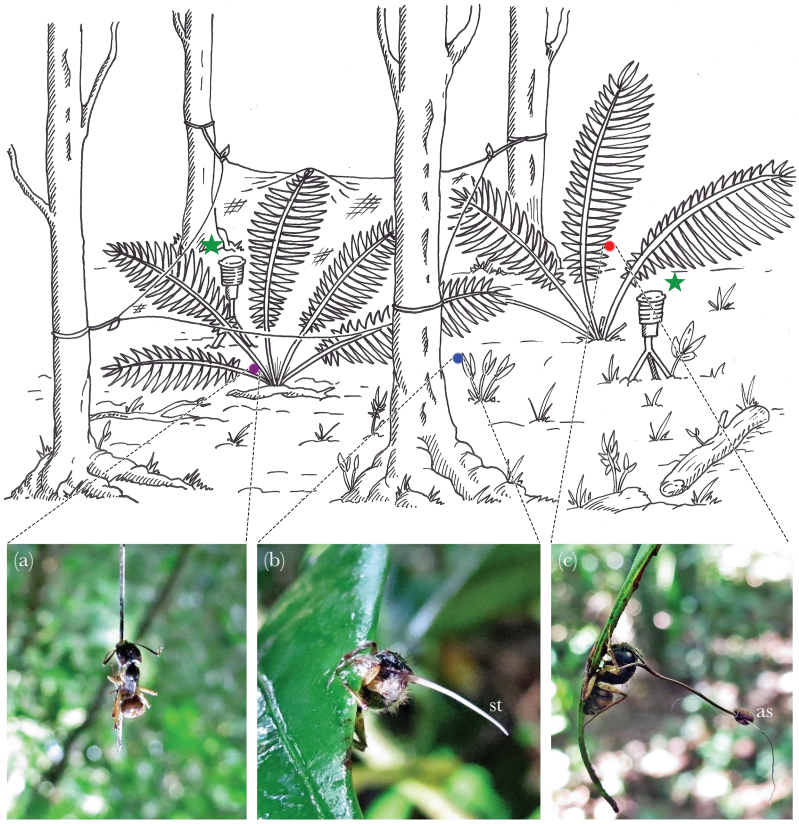

Figure 1
Graphic representation of the experimental field setup for each plot. The purple circle represents a dead ant in shaded region of the plot. The blue circle shows a dead ant in border and the red circle shows a dead ant in control region of the plot. The green stars shows the sensor towers collecting data in shaded and control locations. Photos underneath the graphic representative plot: biting substrates and stages of fungal development emerging from the bodies of dead, infected ants. Recently died *C. atriceps* (less than 72 h) with mandibles clasping a palm spine, there is no external fungal growth (a). Dead *C. atriceps* with mandibles clasping a leaf. A stroma (st) is emerging from between the head and pronotum (approximately 1 week after the ant's death) (b). Dead *C. atriceps* with mandibles clasping the main vein of a leaf, showing a stroma with a developed ascoma (as) (i.e., fruiting body) (1–2 weeks after death) (c).

in the kit) were added, prior to incubation in a water bath for 12 h at 60 °C. After incubation, we followed the Plant Mini kit protocol as per the instructions.

The regions nuc-LSU (nuclear large subunit rDNA) and RPB2 (RNA polymerase II second largest subunit) were amplified using an Applied Biosystems Veriti 96 Well Thermal Cycler, and the previously reported primer pairs LR0R/LR5 and RPB2-6F/RPB2-7R, respectively (White et al. 1990; Liu et al. 1999). PCR reactions were performed using 1U Platinum® Taq DNA Polymerase, 1× PCR Buffer and 1.5 mM $MgCl_2$ (Invitrogen), 3 mM of each dNTP (Promega Corporation), 2 µM of each primer, and 3µL of DNA at 5 ng/µL. The PCR program for nuc-LSU amplification consisted of initial denaturation at 95 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and then a final extension step at 72 °C for 10 min. The RPB2 region was amplified as reported by Liu et al. (1999). PCR fragments were visualized using a 1.5% agarose gel stained with GelRED™ (Biotium), and ultraviolet light.

Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems), according to the manufacturer's instructions. Sequencing was performed with an Applied Biosystems ABI 3130 Sequence Analyzer, automated for capillary electrophoresis. Sequence quality visualization, consensus sequence assembly, and alignment were performed using Geneious R9 (Kearse et al. 2012). Sequence and species identity confirmation were performed using NCBI BLASTn.

Statistical data analysis

Abiotic variables were analyzed using Generalized Linear Mixed Models (GLMMs), with dependent variables being mean light intensity, air temperature, and relative humidity at each hour of the day. The treatment (shaded area vs. control) was the fixed predictor variable, and plots were considered random variables. To test GLMM fit, we compared the Akaike's Information Criterion (AIC) of each GLMM with the AIC of its respective null model (intercept and random variable only). A complete GLMM was accepted when its delta AIC (difference between the complete and null model) was greater than 2 (Akaike 1982). All GLMMs were created with the lme4 package (Bates et al. 2015) of R version 3.2.3 (R Development Core Team 2016).

We used paired *t*-tests to compare the number of new ant cadavers, and the number of fruiting bodies produced during the 6-month monitoring (dependent variables) in the shaded and adjacent control areas (predictor variable). For this analysis, the plot was considered the sampling unit.

The effect of incident light intensity on cadaver height was also investigated using a GLMM. The dependent variable was

cadaver height above the forest floor. Fixed predictor variables were the treatments (shaded vs. control area), and the position of the cadaver in the areas (area border vs. interior). For this analysis, the sample unit was the ant cadaver, and individual plots were considered as random variables to control for spatial dependence in the data. We also ran the same analysis using the height above the forest floor of the ants that were already present in the plots before the experiment setup, was dependent variable. Residual analyses were performed to test model adequacy. For each predictor variable, partial graphs were produced using the visreg package (Breheny and Burchett 2017) in R version 3.2.3 (R Development Core Team 2016). Such graphs show the effect of one variable, with the others being held constant (Breheny and Burchett 2013). In addition to estimating relationships between variables, this package was used to create 95% confidence intervals.

RESULTS

Fungal parasite identification

To identify the fungal parasite in this study, we obtained nuc-LSU (792 bp, Genbank MH469161) and RPB2 sequences (682 bp, Genbank MH476447) that were aligned to deposited sequences in the Genbank database using NCBI's BLASTn. The nuc-LSU sequence showed 85% similarity (random correspondence probability e-value = 0) with the deposited sequence for *Ophiocordyceps sp.* JA-2017b nuc-LSU (Genbank accession number, KX520652). The most similar RPB2 sequence found was for *Cordyceps* sp. OSC 110997 (Genbank accession number, EF468929) with 85% similarity (e-value = 0). The result of the similarity analysis for nuc-LSU (85%) and RPB2 (85%), the species-specificity of the parasite–host interaction between the ant (*C. atriceps*) and the fungus (*O.camponotiatricipis*) (Evans et al. 2011; Loreto and Hughes 2016; Araújo et al. 2018; Sakolrak et al. 2018), combined with the fact that collections were carried out in the same region as the type specimen (Araújo et al. 2015), suggest the identification of the fungus as *O. camponotiatricipis*. Although the possibility of being a new species of fungus cannot be discarded.

Local climatological data

Values of incident solar radiation (µE) were consistently higher in control areas between daily sunrise and sunset ($P < 0.001$). The greatest discrepancies in solar radiation values between the shaded and control areas occurred around solar noon, when overall solar incidence was greatest (Figure 2a). Temperature values were relatively similar between treatments ($P > 0.05$) and varied between 20 °C and 33 °C between day- and night-time (Figure 2b). However, relative air humidity was generally higher and more stable in shaded areas (mean = 95.63% ± SD = 4.07) than in control areas (91.83% ± 7.04) over the daily course of 24 h ($P < 0.001$) (Figure 2c).

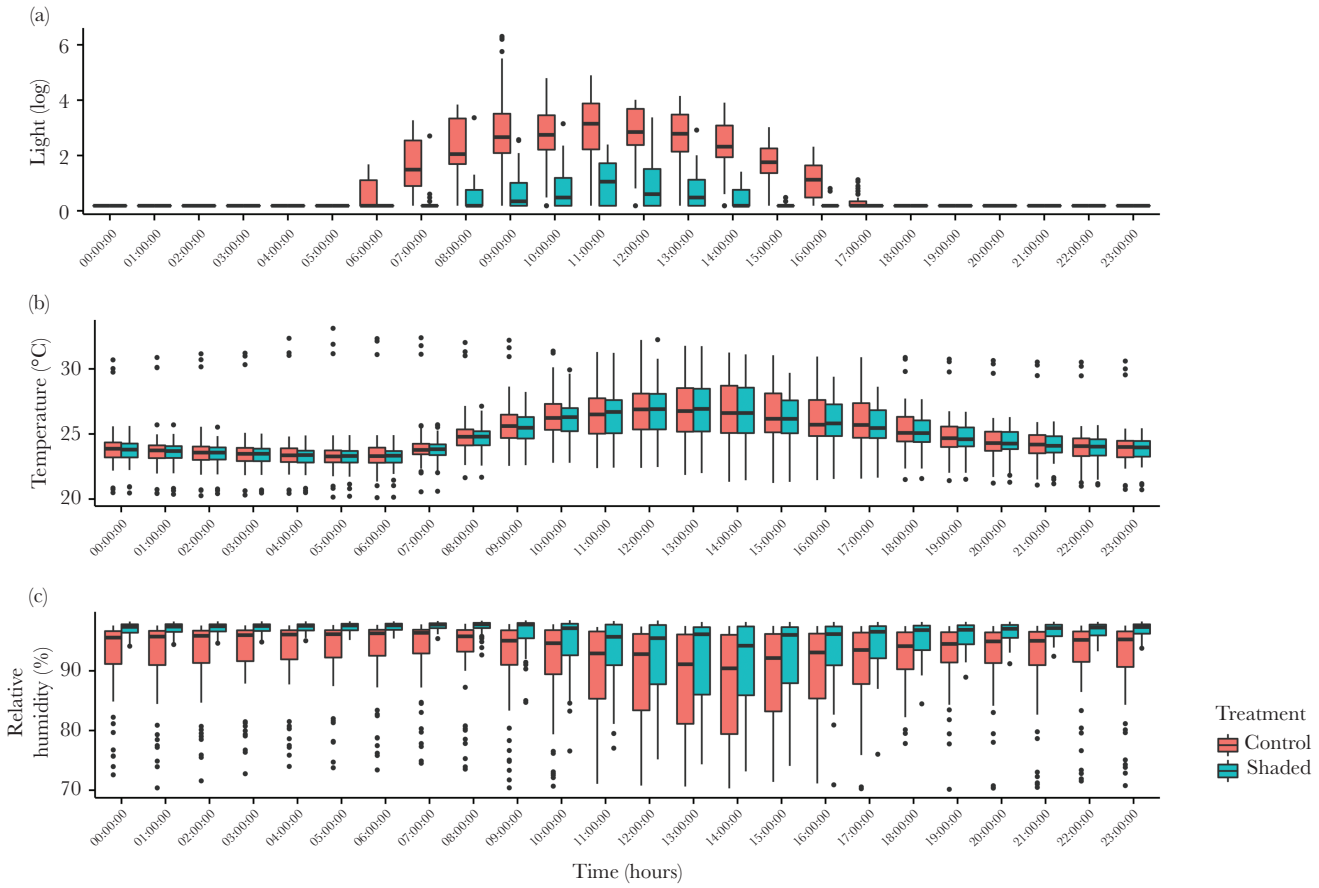

Figure 2
Hourly comparison of recorded climate variables for control and experimentally shaded areas: (a) incident solar radiation (uE), (b) temperature (oC), and (c) relative humidity (%).).

Illuminance and zombie-ant occurence

Newly infected dead ants appeared in 6 of the 10 monitored plots. As a consequence, analyses given here are based on 6 sample pairs. During monitoring, 162 infected dead ants were found: 109 (67.3% of the total) in control areas, and 53 (32.7% of the total) in experimentally shaded areas. Of those 53 ants that died in shaded areas, only 6 died in the central region of the plots and 47 died within the border region where there is more incident light in the early mornings and late afternoons. The number of ants that developed fruiting bodies (56 in total) was also higher in control areas (41 of 109, 73.2% of the total) than in shaded areas (15 of 53, 26.8% of the total). In each individual plot, more infected ants with fruiting bodies were found in the control areas versus the shaded areas (Figure 3a, $t = 2385$, $P = 0.031$, and Figure 3b, $t = 2538$, $P = 0.026$, respectively). Additionally, all 15 ants, from which fruiting bodies emerged in the shaded areas, were found within the 50 cm buffer zones at the borders.

Height of dead infected ants

The height at which infected ants died also showed differences between control and shaded areas (Delta AIC = 26.58). Within the interior part of the areas, on average, ants died at ~110 cm in control areas and ~140 cm in experimentally shaded areas. The height at which infected ants died was also lower in the border regions of the control (mean ~60 cm) compared to shaded areas (mean ~90 cm). Taken together, infected ants died at a mean height of ~80 cm in control areas, versus ~113 cm in shaded areas, regardless of their X, Y coordinates within those areas. Both variables (treatment and position) were found to be relevant for the model ($P < 0.001$, Figure 4). The height above the forest floor of infected ants already present in the plots (before the monitoring) was similar between treatments ($P = 0.55$), with a mean of 91.93 cm in natural areas and 91.57 cm in shaded areas.

DISCUSSION

The place of death of carpenter ants infected with *Ophiocordyceps unilateralis* s.l. is determined at the time the ant's jaws clasp the vegetation in a final'death grip'. The specific location where this behavior occurs defines the last lifetime act of the infected ant. The manipulated biting behavior, along with the ant's search for sites with specific temperature and moisture requirements that benefit the fungus, are considered the parasite's extended phenotype manifesting in the host (Andersen et al. 2009). Our results suggest that light plays an additional, determining role in the behavioral manifestation of the fungus' extended phenotype. Areas where the incident radiation was experimentally reduced contained significantly less *C. atriceps* individuals infected by *O. camponoti-atricipis* than unshaded, control areas. Moreover, the majority of manipulated ants that ended up in the shaded areas did so at the borders where incident light levels are higher in the early morning and late afternoon. The height of cadavers recorded in our paired plots also showed significant differences associated with light intensity reduction. Dead ants were found higher up on the vegetation in shaded areas, than in unshaded control areas, suggesting positive phototropism. These differences are likely the result of the light experimental manipulation, given that the height above the forest floor of infected ants already present in the plots were similar between treatments. This indicates that manipulated *C. atriceps* do not randomly end up at any elevated position, but perceive, and are positively influenced by light intensity when they wander and climb towards their final biting locations. This suggests that the high density of dead ants in graveyards might be attributed to the incident light in these areas, which may be most favorable for fungal development and reproduction. Illuminance levels thus appear to play a role in the manifestation of the extended phenotype of the parasite *O. camponoti-atricipis* in *C. atriceps* ants.

In Thailand, parasitized *C. leonardi* have been found to latch themselves onto vegetation at sites where temperature and humidity fluctuations were minimal. Manipulated ants consistently died at about 25 cm from the forest floor where the temperature was lower and the relative humidity higher compared to the canopy above. These conditions were predicted to favor the development of the fungus (Andersen et al. 2009). In contrast, our results from the Central Amazon in Brazil, indicate that light, is the main environmental driver behind the final summiting behavior of infected *C. atriceps*. While the thermal amplitude between shaded and control areas did not show any notable differences, the relative humidity was higher and more stable in the experimentally shaded areas. Shaded areas also harbored about half of the incident solar radiation (μE) compared to control areas. However, the number of infected ants and the number of developed fruiting bodies were significantly higher in the control areas, where relative humidity levels

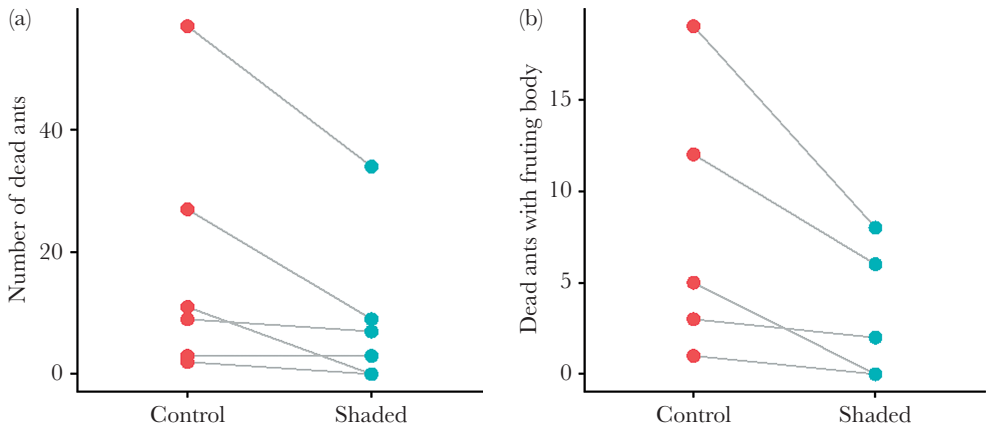

Figure 3
Number of dead ants (a) and number of ants with fungal fruiting bodies (b) in control (red) and shaded (blue) areas. Each pair of circles connected by a line represent the 6 study plots that contained new dead ants registered during the 6-month monitoring.

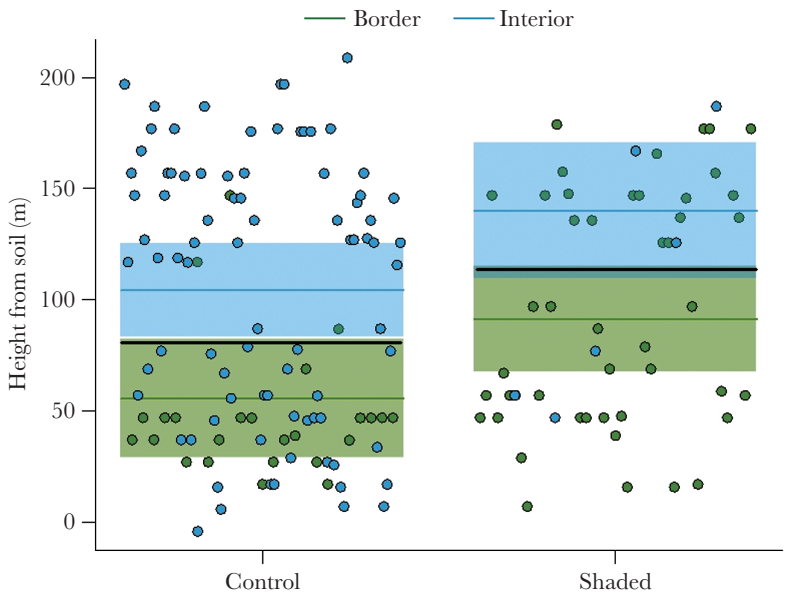

Figure 4
Height of ant cadavers attached to vegetation in shaded and control areas, distinguishing between cadavers found within the interior of the areas (in blue) and the border (in green). Each point represents a dead ant. Green and blue horizontal lines indicate average height for cadavers within borders and interiors of the areas, respectively. Black horizontal lines indicate average height of cadavers throughout the entire areas.

were lower and fluctuated more. While the role of humidity cannot be completely ruled out, these results suggest that, for infected *C. atriceps,* illumination levels represent an environmental variable with greater influence on death site choice than relative humidity. This is somewhat in line with the findings of the field study investigating time of day effects in manipulated *C. leonardi* in Thailand. Here, light seemed to influence and synchronize the timing of the final "death grip" since all investigated ants bit down into vegetation around solar noon (Hughes et al. 2011).

During monitoring, more newly manipulated *C. atriceps* dead ants were found in the control areas than the shaded areas. Ants in these shaded areas were found at overall higher elevations than in unshaded control areas. This suggests that infected ants that ended up in shaded areas right before the final "death grip" continued searching for areas of greater light incidence by summiting to higher elevations. Moreover, the vast majority (47 out of 53) of those ants ended up in the border regions where incident light is higher around sunrise and sunset. Our results, thus, indicate a strong positive phototactic influence on the behavior of infected ants. This is similar to reports on baculovirus-infected caterpillars (Van Houte et al. 2015) and parasitized gammarids (freshwater crustaceans) (Ponton et al. 2006). Baculovirus-infected caterpillars exhibit hyperactivity behavior that results in death at elevated positions on top of the vegetation (Evans 1986). This behavior is adaptive to the virus while, at higher elevations, particle dispersal onto conspecifics is increased as well as trophic transmission towards predatory birds (Entwistle et al. 1993). This behavior is triggered in response to light: infected caterpillars become positively phototatic (Van Houte et al. 2015). Similarly, intermediate gammarid hosts that are parasitized by trematodes, summit to the water surface (behavior that uninfected gammarids avoid) where they are more exposed to light and predators, which act as the parasite's ultimate hosts (Ponton et al. 2006). The median height of dead ants found in the border areas of our plots corroborate the phototactic hypothesis; they are found at lower heights (~90 cm, Figure 4) compared to ants found within the interior areas of shaded plots (~140 cm, Figure 4). This suggests that lateral solar incidence indeed has an influence in such regions.

The fungal reproductive phase (i.e., formation of the fruiting body) also appears to be depending on incident light levels. Only a low proportion of ant cadavers produced ascomas in the shaded areas (i.e., 26.8%, compared to 73.2% in control areas). In addition, the 15 ants from which fruiting bodies emerged in the shaded areas were all located at the borders and, therefore, more exposed to lateral illumination during dusk and dawn. The influence of incident light on fungal reproduction has been extensively studied in the ascomycete *Neurospora crassa*, where light is responsible for the induction of sporulation and sexual development (Ballario and Macino 1997; Schwerdtfeger and Linden 2001). The molecular photoreception mechanisms underlying our results may very well be similar to those discovered in *N. crassa.* Investigation into the molecular pathways triggered by light during extended phenotype manifestation thus promises to be an interesting and important avenue of research to unravel the inner workings of parasitic behavioral manipulation (de Bekker et al. 2014; Chung et al. 2017; de Bekker, Will, et al. 2017).

The results of the study presented here, augment evidence for the role of circadian rhythms in the precise control of ant behavior mediated by fungal parasites. Infected ants only displayed the manipulated biting behavior in the lab when exposed to 24-h light and temperature cycles (de Bekker et al. 2014). Field evidence on *C. leonardi* in Thailand also pointed to high levels of temporal synchrony for the death grip schedule: focal observations recorded ants biting leaves at solar noon and dying late in the afternoon, close to sunset (Hughes et al. 2011). The difference in timing between the lab experiments and field observations could be due to species-specific and local adaptations. However, the shift in timing could also be a result of differences in the strength of entraining environmental cues (i.e., light and/or temperature) between the lab and the field. If the latter appears to be the case, this would be yet another

argument for the role of circadian clocks in the *Ophiocordyceps* infection and manipulation of ants. Ants of the genus *Camponotus* are mostly nocturnal in their foraging behavior (Levings 1983). However, our results indicate that infected *C. atriceps* death sites are influenced by light incidence. The greater number of cadavers and fruiting bodies found in the naturally illuminated control areas suggest a fungus-induced change in the behavioral activity schedule of infected ants. This to ensure manipulated biting during the daytime, during which the ant can be navigated to seek a location with incident light levels that are beneficial to the fungal parasite.

The role of illumination, photoreception, and light-entrained circadian rhythms in the biology of insect-infecting fungi is still an underexplored subject of study. While the same can be said for truly linking phototaxis to observed changes in behavior in invertebrates, seemingly light-related examples of parasite-mediated behavioral manipulation are well-documented (Moore 2002). Studies linking molecular mechanisms and environmental physical cues are needed to advance our knowledge concerning the relationships between parasitic fungi and their hosts. In addition, more detailed studies on other *Ophiocordyceps* species may also show the influence of light on their extended phenotypes in order to enhance reproductive success. The results presented here serve as an important reference for such future studies into the influence of incident light on the reproductive success of parasitic fungi of the *O. unilateralis s.l.* complex.

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by Sarti Andriolli et al. 2019.

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