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Natural History Note

The Life of a Dead Ant: The Expression of an Adaptive Extended Phenotype

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ABSTRACT: Specialized parasites are expected to express complex adaptations to their hosts. Manipulation of host behavior is such an adaptation. We studied the fungus *Ophiocordyceps unilateralis*, a locally specialized parasite of arboreal *Camponotus leonardi* ants. Ant-infecting *Ophiocordyceps* are known to make hosts bite onto vegetation before killing them. We show that this represents a fine-tuned fungal adaptation: an extended phenotype. Dead ants were found under leaves, attached by their mandibles, on the northern side of saplings ~25 cm above the soil, where temperature and humidity conditions were optimal for fungal growth. Experimental relocation confirmed that parasite fitness was lower outside this manipulative zone. Host resources were rapidly colonized and further secured by extensive internal structuring. Nutritional composition analysis indicated that such structuring allows the parasite to produce a large fruiting body for spore production. Our findings suggest that the osmotrophic lifestyle of fungi may have facilitated novel exploitation strategies.

Keywords: carpenter ants, histological cross sections, life-history evolution, *Ophiocordyceps*, sclerotia, behavioral manipulation.

Introduction

Specialization in parasite-host interactions often requires adaptations that establish a sustainable level of host exploitation (Combes 2001, pp. 86–87; Poulin 2007, pp. 62–63). Parasite control of host behavior is a prime example of such adaptations (Poulin 2000; Moore 2002). Because of its highly complex nature, behavioral manipulation is often interpreted as being adaptive for the parasite and detrimental to the host (the manipulation hypothesis, see Thomas et al. 2005). Noteworthy examples are crickets committing suicide (Thomas et al. 2002) and spiders constructing postmortem resting structures for their parasitoid wasps (Eberhard 2000). These behaviors are considered to be extended phenotypes of the parasite because aberrant host behavior can be parsimoniously explained as an expression of parasite genes in the host tissue, for the sole purpose of increasing parasite fitness (Dawkins 1982). However, the extended phenotype interpretation is only sound when altered host behavior can be demonstrated to serve parasite rather than host fitness (see discussion in Thomas et al. 2005), and data providing such unequivocal evidence are relatively rare (but see Biron et al. 2006; Grosman et al. 2008; Yanoviak et al. 2008).

The "death grip" of ants infected by the fungal parasite *Ophiocordyceps* is one of the most dramatic examples of inferred parasite manipulation (Bequart 1922; Evans and Samson 1982). Infected ants bite onto vegetation (leaves, bark, stems) in tropical forests just before being killed by the fungus. The parasite then grows a spore-dispersal structure from the base of the ant's head (Evans 1989; video 1 in the online edition of the *American Naturalist*). *Ophiocordyceps* species are specific in both host choice and death location. *Ophiocordyceps kniphofioides* is specialized on *Cephalotes* ants that bite onto bark when infected, *Ophiocordyceps australis* infect ponerine ants that bite and cling onto twigs, while the focus of this study, *Ophiocordyceps unilateralis*, infects *Camponotus* ants that bite onto the undersides of leaves (Evans and Samson 1982, 1984).

By viewing the death grip within the framework of the extended phenotype paradigm (Dawkins 1982; Poulin 1995; Thomas et al. 2005), we set up a number of hy-

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Video 1: From the BBC series *Planet Earth*. Still photograph from a video (MPEG, 3.7 MB) showing time-lapse sequence of *Ophiocordyceps* growth from a ponerine ant.

potheses. An important consideration in generating these is that entomopathogenic fungi such as *O. unilateralis* are distinct from nonfungal parasites because reproductive success is realized after host death (Agrios 1997; White et al. 2003). We therefore hypothesized that *O. unilateralis* has evolved a syndrome of stepwise sequential traits to achieve its reproductive success, beginning with manipulation of the host into dying in an optimal location for fungal development, followed by colonization and exploitation of the dead host body in a way that secures the resources in competition with the abundant decomposers found in tropical forests. We tested this by determining the narrow manipulation zone of dead infected ants and characterizing parasite development in this zone with experimentally relocated hosts as controls. Further, we reconstructed *O. unilateralis* host colonization by combining semithin histological cross sections with nutritional physiology techniques, which revealed highly complex fungal structuring in the dead ant bodies.

Material and Methods

Fieldwork took place in Khao Chong Wildlife Sanctuary, Thailand (7°32′49.50″N 99°47′14.73″E), in September 2006 and 2007. The forest is an evergreen primary forest with very sandy soil. The canopy rarely reaches more than 30 m, and the understory has many saplings of a uniform size. During fieldwork in 2006, five high-density areas with many dead *Ophiocordyceps unilateralis*–infected ants were located (Pontoppidan et al. 2009). These ant graveyards were still active in 2007 and were the primary focus of the sampling efforts in this study. We searched for healthy ant trails and colonies at and around the high-density areas for many hours, at night and during the day.

Three squares (2 m × 2 m) were arbitrarily set up within graveyard areas. For all dead infected ants within these areas, we registered the species identity of the ant and its length, the height of the dead ant above ground, the total height of the plant, the placement of the dead ant on the leaf (midrib, side vein, or elsewhere), and the leaf's compass orientation relative to the plant stem. This was done for 51 host ants of *Camponotus leonardi* and six of a *Polyrhachis* species, which is a minor secondary host of *O. unilateralis* at this site (3% prevalence; Pontoppidan et al. 2009). To increase the sample size for this secondary host, we located 15 additional infected individuals of *Polyrhachis* in the same area. These *Polyrhachis* ants belonged to at least three different morphospecies that were not further identified.

To determine the importance of dead host location for *O. unilateralis* growth, we relocated infected ants after host death. We removed 16 dead *C. leonardi* ants from their leaves and placed them in a marked area directly on the ground. We chose newly dead ants with actively growing hyphae (typical for ants that have been dead for 24 h up to a few days) because this stage is most sensitive to fluctuations in temperature and humidity. Seven uninfected ants were collected from a foraging trail, killed by freezing, and placed next to the dead infected ants to examine what happens to dead uninfected ants placed at ground level. The presence and condition of the relocated ants were recorded daily until they disappeared. Seven newly dead ants were collected with their supporting leaf and placed on a wooden mounting board secured below a metal plate to provide protection against rain and falling debris. The entire construction was placed close to a tree trunk 15 m above ground near a *C. leonardi* nest. The samples were checked and photographed 1 and 2 weeks after relocation. Using the same line system that towed the platform into the canopy we also measured humidity and temperature for 20 min (after 10 min of stabilization of the hygrometer) at ground level, 30 cm above ground, 1 m above ground, and at 5 m, 10 m, 15 m, 20 m, and 25 m. The measurements were performed between 0900 and 1500 hours, both from the ground level upward and the other way around.

We further marked 24 infected ants (22 *C. leonardi* and 2 *Polyrhachis* spp.) that were discovered within 24 h after death and photographed them every day in situ at noon for the next 9 days. The photographs were placed in categories of time since death, and this was used to characterize the typical external fungal growth. The length of the stroma (aerial fungal structure growing from the base of the ant head on which spores are produced) was estimated from the photographs knowing the mean length of a dead infected ant (mean length of 5.63 ± SE 0.13 mm, $n = 51$).

To study host use after infection, we sampled 14 ants

over 2 weeks in the following three categories: dead less than 48 h, dead between 48 h and 9 days, and dead for longer than 9 days and with perithecial plates (spore-producing bodies on the stroma). Individuals were classified on the basis of the level of external fungal growth as defined from the individuals that we followed for 9 days. All ants were processed in the field lab within 5 h of sampling by taking them from their leaves and removing the head to allow penetration of the fixative glutaraldehyde in sodium cacodylate buffer for 24 h. Subsequently the ants were washed in sodium cacodylate buffer and stored at 4°C in fresh buffer. The heads were postfixed in 2% osmium tetroxide and dehydrated in a graded acetone series before embedding in araldite and sectioning with a Reichert Ultracut E microtome in semithin 1-μm sections. The sections were stained with methylene blue, photographed under an Olympus BX-51 microscope connected to a PC, and edited in Photoshop.

We also did scalpel sectioning of infected ants that had been dead for more than 2 weeks. In these cases we always observed characteristic black, orange, and white fungal structures in predictable locations. To analyze the compositional differences between these three types, each of them was dissected out from 20 ant hosts, pooled, and subjected to C : N analysis. All material was dried overnight in a vacuum oven at 60°C and kept in a closed plastic box with silica gel during transportation to the scale to avoid hydration of the samples before weighing. The samples were then quickly transferred to a preweighted tin cup using a funnel and brush and reweighed in a closed weighing chamber with silica gel. The C : N ratio was measured using a dry combustion analyzer (Na2000, Carlo Erba, Italy).

Statistical analysis of the data was performed in SPSS 11.0.4 for Mac OSX (SPSS, Chicago, IL). Humidity and temperature data were not normally distributed, so nonparametric tests were performed because transformation did not normalize the distributions. Values are given as means ± SE. The leaf orientation data were analyzed with circular statistics in the program PAST 1.80, available as free download at http://folk.uio.no/ohammer/past/.

Results

Location of Healthy Camponotus leonardi *Ants*

In total, three trails were discovered at ground level during 3 months of fieldwork. One colony was located in the canopy at ~15 m height and collected by rope climbing (Ellwood and Foster 2001). The ants nested in excavated galleries in a tree stem, and thousands of individuals, including sexuals and brood but no queen, were collected from the nest. Multiple ant trails extended from the colony, crossing the canopy on lianas and branches.

Location of Dead Host Ants and External Ophiocordyceps unilateralis *Development*

The death grip occurred in very precise locations. Biting ants were all on the underside of a leaf, and 98% were found on a leaf vein (63% on the midrib and 35% on a side vein), while 2% were biting a leaf margin. The location of dead ants on leaves was significantly biased toward the north-northwest side of the plant, with a mean angle of 345° (*C. leonardi* host ants only, Raleigh's test: $R = 0.317$, 95% confidence interval [CI] = 314.7°–375.5°, $P \leq .01$; fig. 1*A*). The *C. leonardi* hosts were found close to the ground at a mean height of 25.20 ± 2.46 cm (95% CI = 20.38–30.02 cm; $n = 51$). The infected *Polyrhachis* ants were found at a mean height of 78.43 ± 10.51 cm (95% CI = 57.83–99.03 cm; $n = 21$), which is significantly higher than the *Camponotus* ants, both for the median (Mann-Whitney *U*-test: $U = 135.0$, $P < .01$) and the variance (Levene's test: $F_{1,70} = 26.8$, $P \leq .01$).

Humidity and temperature varied considerably from ground level to the canopy (Kruskal-Wallis test; humidity: $\chi^2 = 250.6$, df = 7, $P \leq .01$; temperature: $\chi^2 = 249.7$, df = 7, $P \leq .01$), and there was a tight negative correlation between them ($r = -1.0$, $P \leq .001$; fig. 1*B*; note the inverse temperature scale). A rather distinct change in environmental conditions occurred at ~1 m: from 0 to 100 cm the humidity was significantly higher and the temperature significantly lower than from 5 m and upward ($U = 665.0$, $P \leq .01$ vs. $U = 34.5$, $P \leq .01$; fig. 1*B*). Not only did the humidity drop and temperature increase from 5 m up to 25 m, but the variation around the mean values also increased (pooled data from 0 to 100 cm compared to pooled data from 5 to 25 m in Levene's tests; humidity: $F_{1,393} = 106.0$, $P \leq .01$; temperature: $F_{1,393} = 69.9$, $P \leq .01$). Even within the narrow band from 0 to 100 cm we found differences, with temperatures significantly lower at 30 cm compared to the soil surface level ($U = 322.0$, $P \leq .01$). Thus, the abiotic environment was very different at 15–20 m in the canopy than where dead infected ants were found <1 m from the ground (fig. 1*B*).

Of the dead ants relocated to the soil surface, all uninfected ants and 14 infected ants disappeared in less than 24 h. The fungus in the remaining two ants grew abnormally and after 1 and 5 days these ants also disappeared. None of the ants that were relocated to the canopy disappeared, but in all cases the fungus grew abnormally, with a distinct gray rather than brown (see below) hyphal growth, which was never seen on dead infected ants under leaves. After the second week all fungal growth on canopy ants had stopped and no stroma developed.

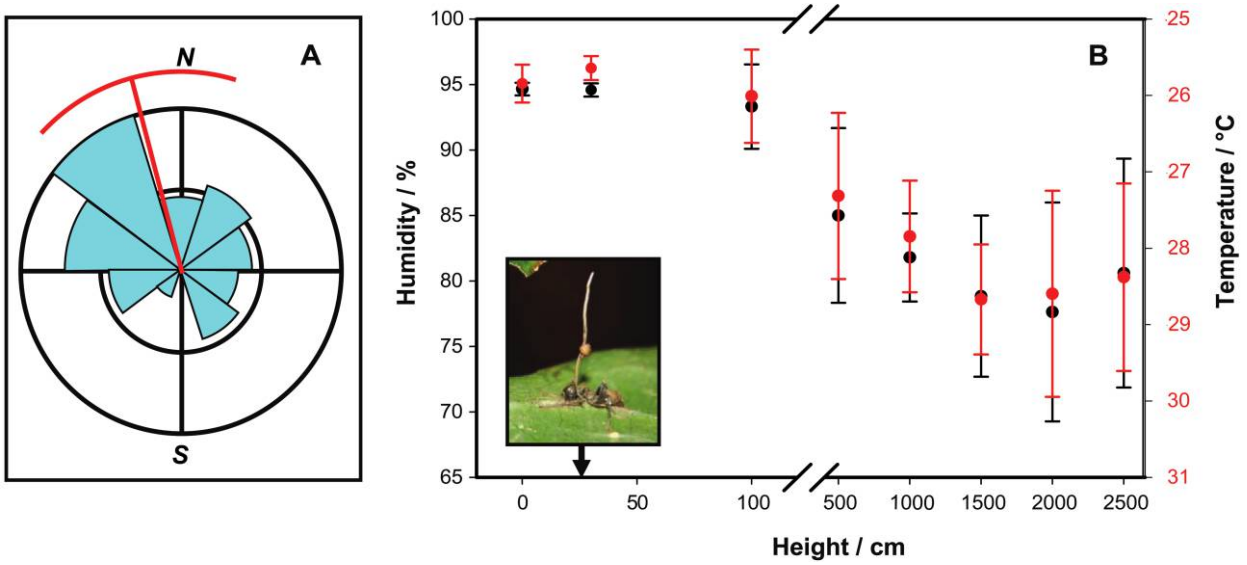

Figure 1: Specifying the spatial abiotic niche of dead ants. *A*, Orientation of dead infected ants relative to the stem of the sapling on which they died. The majority were found on the north-northwest side. The straight red line shows the mean direction at 345.0°, while the red curve marks the 95% confidence interval between 314.7° and 375.5°. *B*, Correlation between humidity and temperature from ground level to the canopy. From 5 m upward, humidity is low and temperature high (note reverse temperature scale), and large fluctuations occur in both. In contrast, from the ground up to 1 m, humidity is high and temperature low. Dead *Ophiocordyceps unilateralis*–infected ants were found at a mean height of 25.20 ± 2.46 cm (*inserted picture and arrow*), whereas dead host ants of the rare secondary *Polyrhachis* host were found at 78.43 ± 10.51 cm.

A characteristic external pattern of fungal growth was reconstructed from the 24 closely studied dead ants (see also video 2 in the online edition of the *American Naturalist*). Within 24 h after death, sparse hyphae were visible growing from the thinner nonsclerotized parts of the cuticle (the intersegmental membranes and the antennal joints), giving the ant a fluffy appearance (fig. 2, *AI*). At the same time, fungal hyphae growing from the tarsae firmly fixed the ant body to the leaf. Infective, asexual spores are produced from these hyphae. The beginning of a white to pinkish stroma was seen developing from the base of the ant head as early as the second day (fig. 2, *AI*). Three to four days after host death the stroma had reached a length of 3–5 mm, and the entire ant and its attachment points to the leaf were covered in a dense mat of hyphae (fig. 2, *BI*; see also fig. 4). These later turned brown, a characteristic color for this *Ophiocordyceps* species. The initiation of sexual reproduction was seen as early as 7 days after host death. This was evidenced by the development of a unilateral perithecial plate on the stroma (hence the species epithet) from which sexual spores are discharged. By this time the stroma had reached a length of ~10 mm (fig. 2, *CI*), almost twice the average length of the *Camponotus* host ants. Thus, external hyphal growth and asexual reproduction occurred in a matter of days, while the transition to sexual reproduction was initiated 1–2 weeks after host death.

Internal Colonization and Occupation of C. leonardi *Tissues by* O. unilateralis

The semithin sections of infected ants >48 h after death showed a high density of fungal tissue with a combination of hyphal growth and yeastlike cells (fig. 2, *AII–AIII*), coinciding with the external appearance of a hyphal matrix. The mandibular muscles were slightly disintegrated but clearly discernible. From 2 to 9 days after death, the internal hyphal growth formed a tightly packed mass, which always emerged from a specific point at the back of the head as the start of the stroma (fig. 2, *BII*). Muscle structures were still recognizable at this stage. There was no evidence of cuticle degradation (fig. 2, *A–CIII*), as both the exoskeleton and the internal cuticular structure that provides support to the head exoskeleton (tentorium) were undamaged (fig. 2, *AIII*). The ants that had been dead for >9 days had a stroma carrying well-developed perithecial plates. We could not determine the exact time since death for these ants, but our extensive field surveys suggest a range between 2 and 8 weeks. Muscle structures were still visible at this stage (fig. 2, *CIII*), but the fungal mass

Video 2: Still photograph from a video (Windows Media, 1.8 MB) showing reconstruction of growth of *Ophiocordyceps unilateralis* infecting a *Camponotus leonardi* ant, followed for 9 days.

seemed reduced and more concentrated in the center of the head cavity. Scalpel dissections of dead ants in this category ($n = 20$) confirmed that most of the head space was occupied by fungal biomass but that hyphae were physically detached from the inside of the head cuticle, a disassociation that was also observed in the gaster (i.e., posterior abdomen) of these ants.

Dissection of entire infected ants that had been dead for more than 2 weeks showed extensive internal structuring of the fungal tissues. We always found a round black structure in the gaster embedded in orange material, which spanned the entire ant from its mandibles via the base of the stroma into the gaster in a tubelike manner (fig. 3). The orange structure was surrounded by white hyphal growth throughout the dead ant, similar to the hyphae visible externally. Chemical analysis of the orange structure showed that it was very rich in carbon compared to the white hyphal growth and the black structure in the gaster. A C : N ratio of 45.7 compared to, respectively, 10.3 and 7.5 (20 individuals pooled) indicates that these fungal structures are likely to have different functions and that the orange material has a specific role in carbohydrate storage.

Discussion

The results reported here provide compelling evidence for a complex, extended phenotype of the *Ophiocordyceps unilateralis* parasite, expressed as a highly specific sequence of behavioral manipulation, growth, and reproduction (figs. 1–3). A tropical rain forest is a structured 3-D space where plants and animals, including ants, occupy and exploit distinct niches (Basset et al. 2003). Our study precisely defines the spatial niche of behaviorally manipulated ants in this 3-D space, at ~25 cm above ground under a leaf on the north-northwest side of a plant, and shows how it is highly distinct from the niche of healthy host ants. Experimental relocation of dead infected host ants outside this niche proved detrimental to fungal fitness, probably reducing it to 0. This confirms that active fungal positioning of hosts in the well-defined manipulative zone enhances parasite fitness.

Strong evidence for parasite manipulation to increase transmission has been found in other parasite-host systems, for example, the water-seeking behavior of hairworm-infected crickets (Thomas et al. 2002; Biron et al. 2006) and attraction of secondary hosts by fruit mimicry in nematode-infected ants (Yanoviak et al. 2008). In these cases parasite growth predominantly occurs while the host is still alive, and host death marks the termination of the association. However, *O. unilateralis* develops inside a dead ant for at least 2 weeks before sexual reproduction is possible. Host death is therefore merely the starting point for the major part of parasite development. In this regard *O. unilateralis* is similar to plant parasitic fungi that develop in their hosts postmortem (termed necrotrophy; see, e.g., Hammerschmidt 2006). Fungi have osmotrophic growth, where excreted enzymes mediate the external breakdown of the substrate by secondary metabolites, followed by uptake of sugars and amino acids. This ability facilitates the shift in *O. unilateralis* from using live tissue to dead tissue, and such a parasite strategy requires exploitation strategies in living hosts that ultimately maximize fitness returns from dead hosts.

Host-Parasite Niche Segregation

Behaviorally manipulated hosts often occupy distinct niches from healthy hosts to increase parasite transmission (Moore 2002). Examples are the above-mentioned movement of hairworm-infected crickets into aquatic habitats (Thomas et al. 2002) and the light-seeking behavior of acanthocephalan-infected amphipods, which move from the secure lake bottom toward the parasite's secondary duck host at the surface (Moore 2002, pp. 37–39). Such segregation is also observed in the *O. unilateralis* system. The colonies of *Camponotus leonardi* were found in the canopy where abiotic conditions were highly variable. In contrast, the dead infected ants were located 25 cm off the ground under leaves, where temperature was consistently low and humidity consistently high (fig. 1). There is ample evidence that humidity levels of at least 94%–95% are required for the development of entomopathogenic fungi, and that temperatures should remain in the range of 20°–30°C because higher temperatures are directly

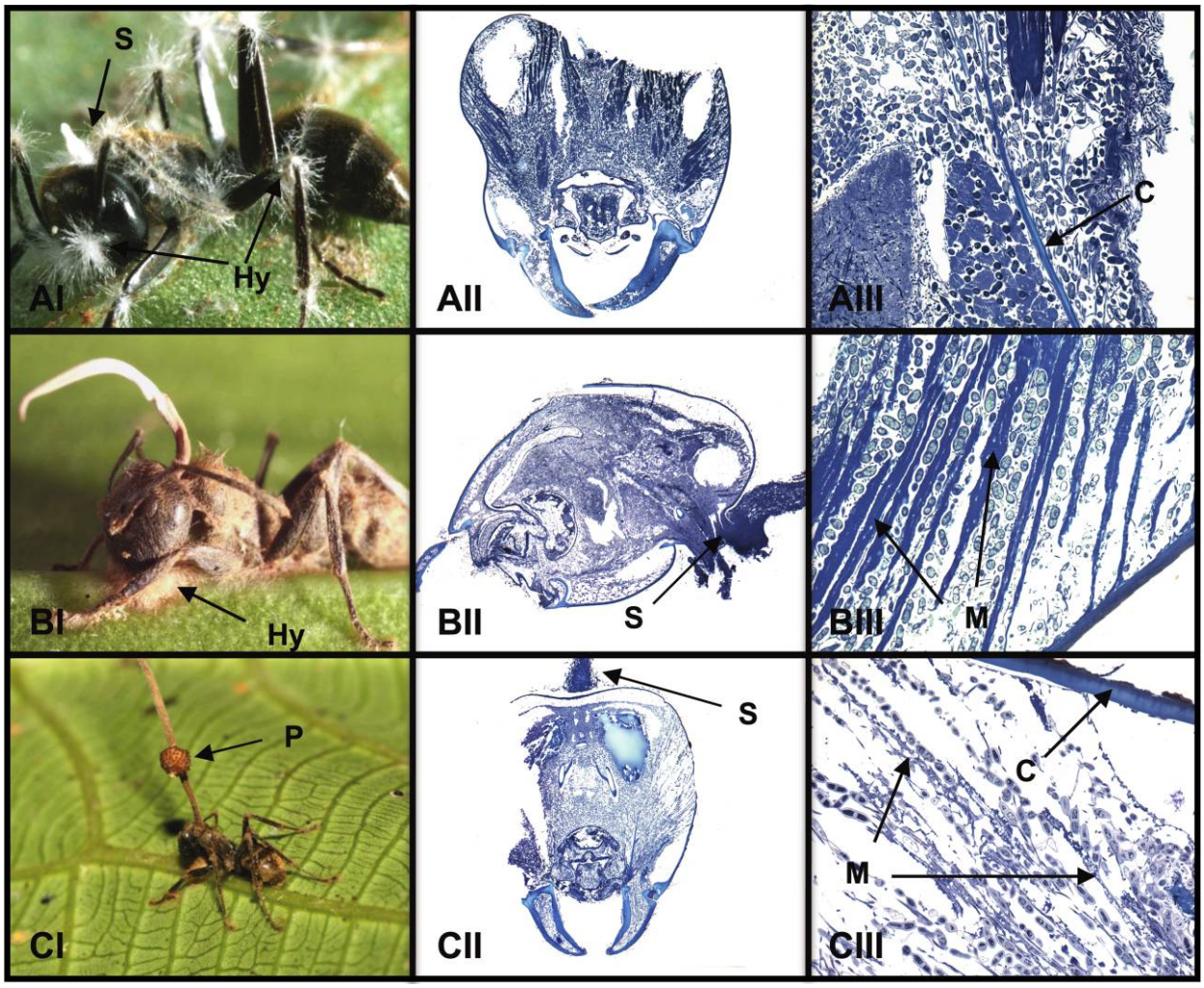

Figure 2: Phenology of *Ophiocordyceps unilateralis* infection in a *Camponotus leonardi* ant host. An ant that has been dead <48 h, with hyphal growth (*Hy*) visible from the intersegmental membranes and antennae. The beginning of a stroma (*S*) is evident at the head base (*AI*). Fungal growth in the head is extensive (*AII*) and includes a mixture of yeastlike cells and hyphae, but the internal cuticular support structures (*C*) are intact (*AIII*). After 9 days, a conspicuous stroma extends from the head base, and the ant and attachment point to the leaf are covered in a matrix of brown hyphae (*BI*). The stroma consists of tightly packed hyphae, continuing partly into the head itself (*BII*). The muscle fibers (*M*) are still discernible at this stage but appear collapsed (*BIII*). A mature *O. unilateralis* stroma (*CI*) with perithecial plates (*P*) a few weeks after host death. The fungal tissue inside the ant head is now detaching slightly from the cuticle (*CII*), but the muscle structure is still partly intact and yeastlike cells positioned between the remaining fibers appear to support the structure; also, the outer cuticle is intact (*CIII*).

harmful (Oduor et al. 1996; Arthurs and Thomas 2001). Small daily fluctuations in humidity are known to trigger spore shooting (Paulitz 1996; Su et al. 2000; Roy et al. 2006; N. L. Hywel-Jones, unpublished data), but more dramatic fluctuations limit fungal growth (Oduor et al. 1996; Luz and Fargues 1997). The canopy appears to be a harsh environment for *O. unilateralis* because all growth ceased in the relocated individuals. The majority of hosts relocated to the ground quickly disappeared, probably because of scavenging or heavy rainfall. In the few remaining, parasite growth was severely affected. They became partially covered in leaf litter and wet sand, which effectively stopped normal growth and prevented successful spore transmission. *Ophiocordyceps* species that infect insects on the ground release light spores from the terminal perithecium for wind dispersal (Hywel-Jones 1996). In contrast, the heavy spores of *O. unilateralis* are actively discharged from the unilateral perithecial plate, and this must take place at some distance from the ground to secure dispersal.

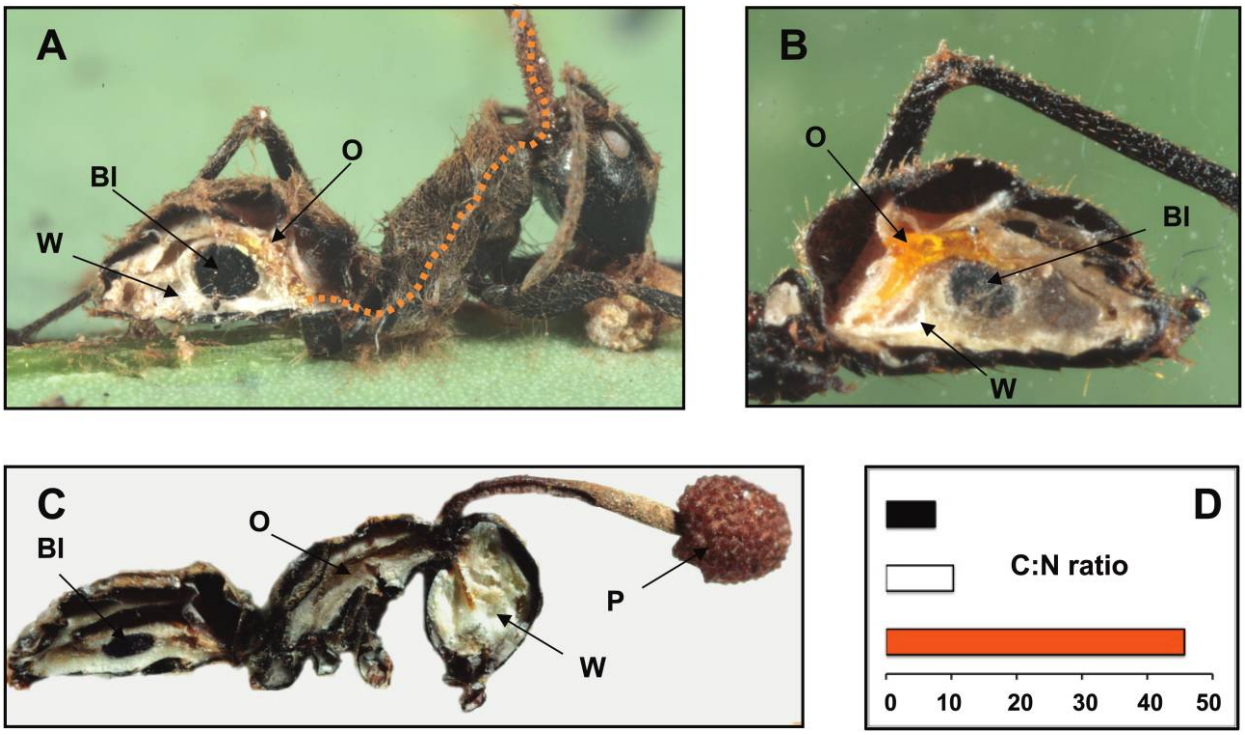

Figure 3: Securing ant resources for future parasite reproduction. Longitudinal dissections of entire *Ophiocordyceps unilateralis*–infected ants (*A–C*) showing an orange structure (*O*) spanning the entire ant and a round black structure in the gaster (*Bl*) embedded in orange material. White hyphae (*W*) cover the orange structure and may facilitate resource transport toward the stroma (*A*). *P* indicates a perithecial plate on the fungal stroma. C : N analysis showed that the orange part of the fungus is rich in carbohydrates, whereas the black object did not differ from the hyphae and could be an endosclerotium formed by densely packed hyphae (*D*).

The precise location of dead infected ants at 25 cm with little variation is remarkable in itself, but ants were also found on specific leaves primarily on the north-northwest side of plants. A similar pattern has been observed in fungus-infected dungflies, which are located against the predominant wind direction (Maitland 1994). Which cues in the *Ophiocordyceps* system mediate this choice and the possible effect on fitness should be further investigated, but solar cues are likely to be important. The rare secondary *Polyrhachis* host ants provide an interesting comparison to the primary host. The infected ants expressed the biting behavior but were not located in as well-defined a zone as the *C. leonardi* hosts. We hypothesize that this difference represents a constraint in adaptation to a rare alternative host. Further work will have to show what causes this difference and whether it affects parasite fitness.

The difference between the canopy niche of the healthy ants and the spore-producing niche of the parasite in dead ants just above the soil surface illustrates the potential costs of parasite specialization. We rarely encountered foraging trails of *C. leonardi* on the forest floor during many months of fieldwork that included both daytime and nighttime surveys. This is, however, where infection must take place, as spores of *O. unilateralis* are actively discharged and dispersed over short distances, creating an infectious "killing field" of ~1 m^2 below the dead host (N. L. Hywel-Jones, unpublished data). Spore release by insect pathogenic fungi typically takes place at night, and spores lose their infectiousness quickly, so that colonization of new hosts has to occur shortly after spore discharge (Oduor et al. 1996; Sung et al. 2007). Transmission possibilities are therefore limited when the primary host species is far away and most other ant species at the forest floor are apparently unsusceptible (based on our surveys). To compensate for this, spore production has to take place continuously over an extended period of time to secure transmission to the occasionally passing *C. leonardi* ant.

Store and Protect: Postmortem Host Exploitation Strategy

Because the association with the dead host extends over a period of time, the host resources must be preserved. The parasite achieves this by manipulating the host ant into securing itself firmly to a leaf by biting into a major

vein (98% of all hosts studied) on the sheltered and shaded underside of a leaf. After host death the ant is rapidly enveloped by hyphal growth, which further secures it to the leaf (fig. 4). The brownish color of the hyphae and stroma derives from red/purple pigments composed of different naphthoquinones with antibiotic properties (Unagul et al. 2005). Also, metabolites with antimalarial and anticancer properties have been isolated from *O. unilateralis* (Isaka et al. 2005). The brown external hyphae may therefore provide protection against invasive microbes that would compete with *O. unilateralis*. The external hyphal colonization only happens from the thin intersegmental membranes, without disrupting the cuticle, while both the exoskeleton of the ant itself and the internal cuticular structures such as the tentorium remain well preserved. The ant cuticle is thus remodeled into a protective case by reinforcing the weaker parts. The subsequent disassociation of fungal mass from the inside of the cuticle that is evident in ants dead more than 2 weeks (fig. 3) reduces contact with potentially invasive spores from the outside even further. The preservation of ant muscles many weeks after death is likewise remarkable and suggests that this tissue plays some functional role in the biology of the fungus.

The sealed-off *O. unilateralis* inside the body cavities of a dead ant expressed a complex internal structuring (fig. 3). We hypothesize that the orange fungal structure, with its high C : N ratio (fig. 3), has an energy storage function. Together with the maintenance of the weaker parts of the insect cuticle, this suggests a "protect and store" resource strategy, required because of the long-term host use. We hypothesize that carbohydrate storage centrally in the dead

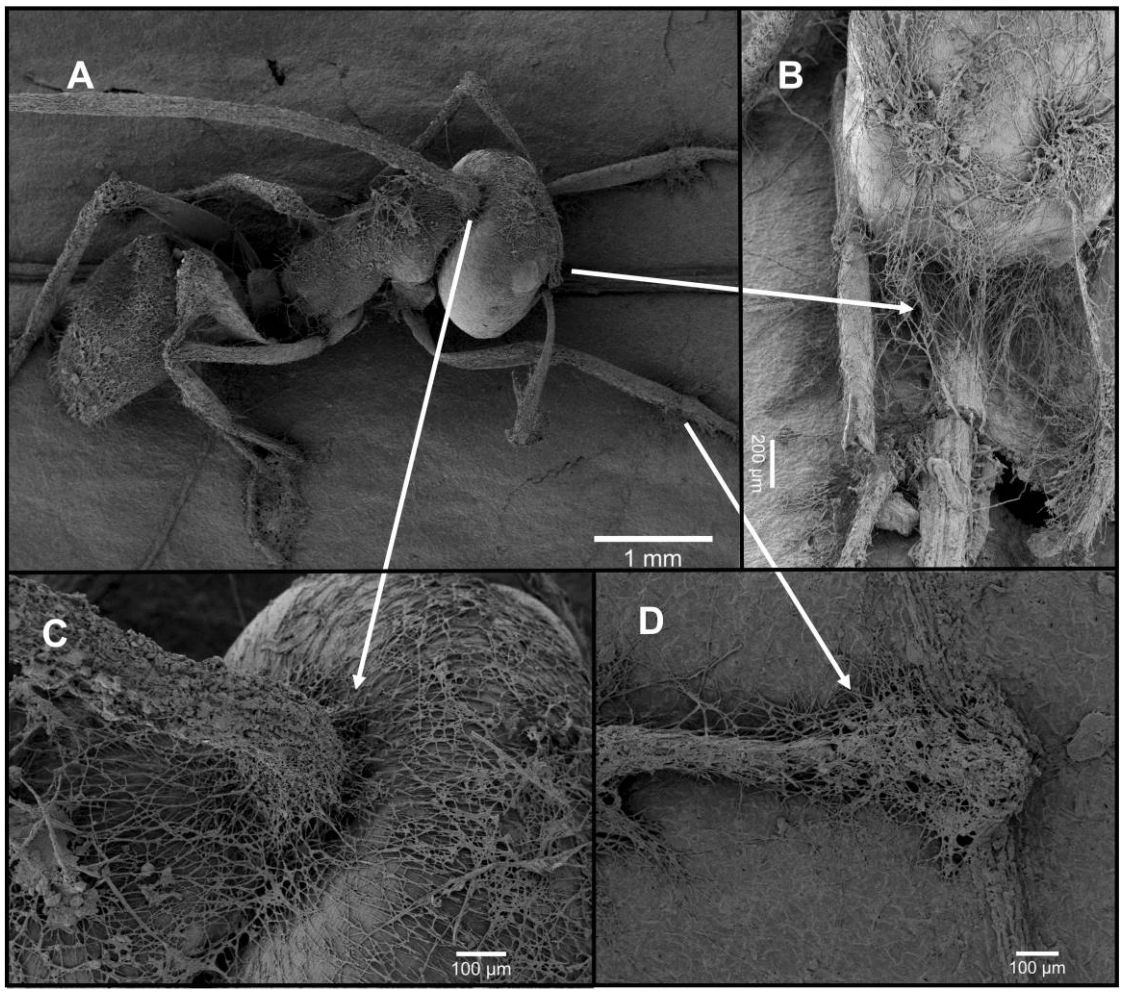

Figure 4: SEM of a dead infected ant. The entire ant (*A*) is covered in a dense matrix of fungal hyphae, securing the biting mandibles (*B*) and the legs to the leaf (*D*). The fungal stroma is penetrating from the basal region of the ant head (*C*).

host could support the continuous spore production from the perithecial plates. The dark mass located distally in the ants is probably a sclerotium formed by densely packed hyphae because its C : N ratio is the same as that of hyphae. Sclerotia are characteristic of certain fungi, where they function as resistant resting structures (Erental et al. 2008). Formation of sclerotia can be induced as carbohydrates and other metabolites accumulate during substrate degradation (Chet and Henis 1975), which is likely to happen inside the ant shell. Whatever the functions of these structures, the high degree of internal structuring is a novel discovery and future work should therefore address these alternative explanations.

The existence of *O. unilateralis*–infected ants has been recognized for a long time (Bequart 1922), but our results provide novel insight into a system with considerable potential for studying adaptive parasite manipulation. Interesting themes to address will be the trade-offs between specialization on a primary host relative to using multiple hosts, and between rapid reproduction with asexual spores relative to later reproduction with sexual spores requiring preservation of the dead host ant. Also, the mechanisms of host manipulation and the actual cues used to affix dying ants to such precise locations deserve further study. Not only is the manipulation strategy of *O. unilateralis* affected by the host nesting biology, but the reverse may also apply. At another site close to where we collected our data, *O. unilateralis* was completely absent and ground trails of *C. leonardi* were observed to be much more common (A. M. Schmidt, personal communication). It would therefore be interesting to test whether host ant foraging behavior and perhaps even nest site location are directly affected by the probability of becoming infected at the ground level.

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Left, measuring the location of dead infected ants (photograph by David P. Hughes); *right*, ant colonies were located >15 m up in the canopy (photograph by Sandra Andersen).