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Azteca ants repair damage to their *Cecropia* host plants

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Abstract
Some *Azteca* ants are well-known symbionts that defend their *Cecropia* host plants against herbivory, although there is considerable variability in behavior among colonies, conditions, and species. In exchange, ants receive food, and also shelter within the plants' internodes. Here we demonstrate that ants repair damage to the host plant when their brood is directly threatened. Using comminuted plant fibers and an unidentified binding liquid (probably plant sap) ants generally began patching holes in the tree trunk immediately, and significantly reduced the size of the hole 2.5 hours after it was created, and they generally completed the repairs within 24 hours.

Keywords
Ant-plant interactions, mutualism, nest construction, nest repair

Introduction

In mutualistic symbioses one partner is expected to be sensitive to danger to another partner if any necessary services (e.g., providing food) are threatened, but to be largely indifferent if not (e.g., Bronstein 2015). Some animals live in shelters made by their symbionts, such as snapping shrimp living within corals (e.g., Chak and Rubenstein

2019), and ants within plants (e.g., Janzen 1967; Longino 1991a; Davidson 2005). In many ant-plant symbioses the ants are provided with food and shelter in exchange for guarding the plants against herbivores, and plants ensure against cheating by linking the food they produce (e.g., Mullerian bodies) to the numbers of surviving leaves (e.g., Davidson 2005). These rewards benefit both partners, given widespread and intense pressure from herbivores, especially on young leaves (e.g., Coley and Barone 1996). But what happens when damage to the plant compromises the ants' shelter instead of its food production?

Some species of *Azteca* ants live in a permanent symbiosis with *Cecropia* plants: these mutualistic ants aggressively defend their host plants against herbivores, and in exchange the plants provide them with food and shelter (Longino 1999a; Davidson 2005; Marting et al. 2018a, b). The ants prevent damage to their hosts' leaves, because the ants' food is produced at the base of the petioles (e.g., Bischof et al. 2013) and the undersides of the leaves (e.g., Folgarait and Davidson 1995). Worker ants also open and maintain small (~1 mm) entrance holes in each internode that they occupy (Fig. 1a).

One of us (AW) used a sling shot to shoot a clay ball (9 mm diameter) at high velocity through an upper internode of a large *Cecropia* tree, making clean entry and exit wounds. Within 24 hours both holes were nearly sealed. This anecdotal observation suggested that the ants may also provide an additional benefit in repairing structural damage to their host plant. We tested the hypothesis that symbiotic *Azteca alfari* ants repair breaches into the interior of symbiotic *Cecropia* host plants that provide them shelter.

Materials and methods

Study area

Observations and experiments were made during the rainy season from June through November 2020. We opportunistically selected smaller trees growing near the edge of disturbed secondary-growth tropical forest (Bosque Urbano de Cárdenas), or located in backyards within the community of Cárdenas, Ancón, Republic of Panamá (~8°59'22"N, 79°34'10"W). Movement and logistics were constrained due to public health restrictions during a pandemic.

Data collection

The size of an *Azteca* ant colony is related to the diameter of the plant stem, and taller trees have more internodes (Marting et al. 2018a). As trees grow the colonies shift their domiciles and broods upward, and abandon lower internodes (*op. cit.*). A DeWalt portable electric drill with a 6.4 mm bit was used to drill a hole into each of $N = 22$ *Cecropia* trees that were all inhabited by *Azteca alfari* ants (Fig. 1b). In all cases the hole was drilled in the center of the internode at heights < 2 m from the ground; we selected internodes as high up as we could reach. Anecdotal preliminary observations

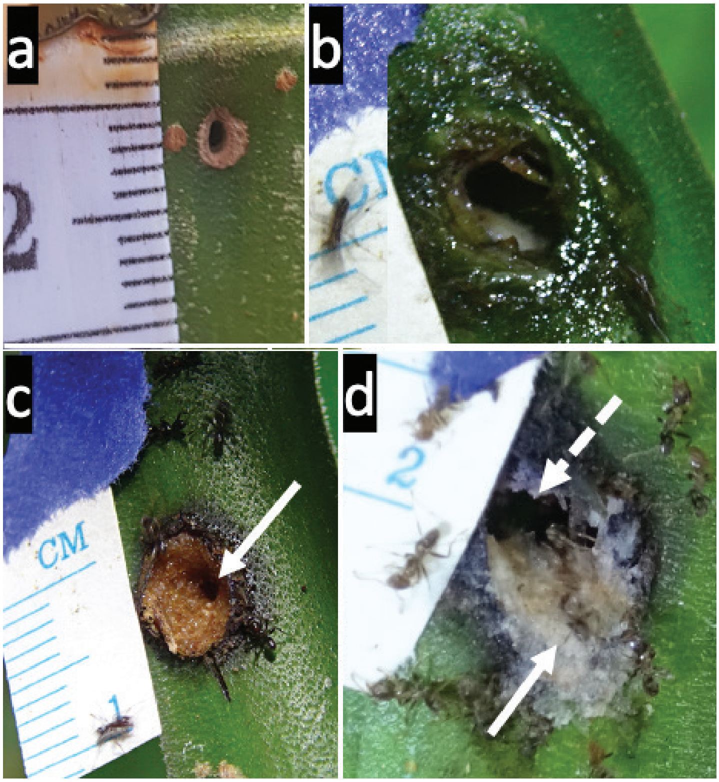

Figure 1. Natural and experimentally made holes in the walls of *Cecropia* stems, exposing the interior of the internode, and repair patches applied by ants (scale bars, mm) **a** a natural entrance to an internode opened and maintained by the ants **b** a newly drilled 6.4 mm hole in a stem (diameter of seventh internode from terminal bud = 19 mm, of 23 internodes total) **c** rapid reduction in hole diameter after 2.5 hours (arrow points to the remaining hole) **d** slower reduction in hole diameter after 24 hrs (dashed arrow points to the remaining hole), with a light-colored patch over half the hole (solid arrow) (diameter of sixth internode from terminal bud = 1 cm, of 50 internodes total).

suggested that ants tended not to respond when holes were drilled into internodes near the base of very large trees, so we used smaller trees, haphazardly selected. We measured the diameter at breast height (DBH), and counted the number of internodes from the terminal bud to where one hole was drilled. To assess whether plants are able to heal themselves in the time frame of our study, we drilled holes in two small myrmecophytic *Cecropia* stems (~1 cm DBH) that had not been colonized yet (not included in quantitative analyses).

Photographs of drilled holes each included a mm scale bar, and were taken with a hand-held Samsung Galaxy S8+, which introduced some non-systematic imprecision (e.g., minor deviations in the angle of the photograph, or a slightly out-of-focus scale bar). Photographs were taken immediately after the hole was drilled (Fig. 1b), after 2.5 hrs (Fig. 1c), and after 24 hrs (Fig. 1d, Fig. 2). These photographs were used to assess the extent to which the holes were sealed by measuring the diameter of the opening. Additionally, we took photographs of natural entrance holes from $N = 8$ trees to determine the diameter of openings that the ants create and maintain (Fig. 1a). Photographs for figures, and Suppl. material 1 and 2, were recorded with a Sony Handycam FDR-AXP35 camera (20.6 MP resolution). Photographs were not digitally manipulated, except for cropping.

Data analyses

Hole diameters were measured from photographs using ImageJ 1.53. Statistical tests are indicated in the Results and were done using packages in R 4.0.3 and R Studio 1.3.1093 (RStudio Team 2020). Many variables did not meet assumptions of normality (Shapiro-Wilk tests) (statistical results given in Text Suppl. material 3), so non-parametric paired statistics were used to test differences in medians. Mean values are given with their standard deviations, and for all paired comparisons $N = 22$. Voucher specimens: Voucher specimens of the ants are deposited in the dry reference collection of the Smithsonian Tropical Research Institute, and were identified as *Azteca alfari* using Longino (1991b).

Results

Cecropia spp. trees in our sample were relatively young and small (mean number of internodes from terminal bud to drill site = 41.7 + 31.5; mean DBH = 3.0 cm + 0.77). Each internode had a natural entrance with mean diameter = 0.76 mm + 0.27, $N = 8$) (Fig. 1a).

Once a hole is opened *A. alfari* ants reacted to the disturbance as described in Marting et al. (2018b), running to the area of the wound. In cases where holes were drilled into internodes that contained brood near the top of small saplings, ants exited with brood and transported them to adjacent internodes (see Suppl. material 1 and 2). The materials used to patch the drill hole are undetermined plant fibers bound by a liquid, presumably plant sap that often was visible around the wound, especially in internodes where the tissue was still green, which continued to ooze after it was sealed by the ants (Fig. 2b). We were unable to determine if ants added any glandular secretions to the patches. Usually several to ~10 ants worked on repairing the hole from both outside and within the stem (see Suppl. material 1 and 2), but ants were not marked so the total size of the repair force is unknown. No ants carried foreign material (e.g., resin) to the repair site (based on ~ 4 hours video observations). Ants carried small pieces of plant material and patched the hole from inside and outside the nest; in the latter case, ants exited from an adjacent internode with building materials (see Suppl. material 1 and 2). Ants applied their pulp to the hole working inwards around the entire circle (Fig. 1c, d). Necrotic tissue around the perimeter of the wound quickly turned black, which was especially conspicuous in small stems that lacked ants (Fig. 2d; these stems were not included in quantitative analyses). Once the hole was sealed (Fig. 2a) they continued to apply plant materials to fill it in

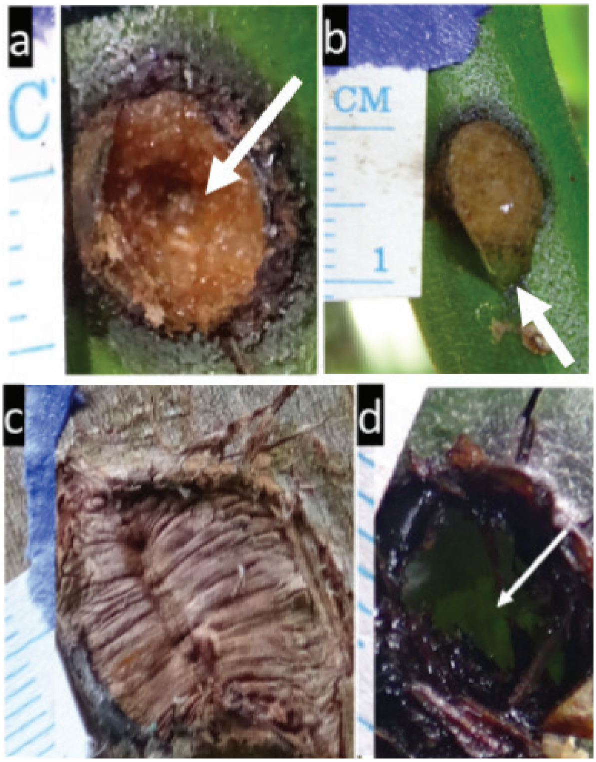

Figure 2. *Azteca* ant and *Cecropia* plant responses to wounds in the stems **a** a sealed hole after 24 hours, but not yet filled in to the stem surface (arrow) **b** a fully patched and filled-in hole after 24 hrs, oozing sap from the ant-sealed wound (arrow) **c** a natural plant scar surrounding a 6.4 mm hole that was fully sealed by the ants, approximately 5 months later **d** a hole in a plant without ants after 24 hrs, showing the green wall of the opposite side of the stem (arrow).

completely (Fig. 2b). Eventually the plant formed its own scar tissue, which differs in color, texture, and durability (Fig. 2c). The initial diameter of the opening (median drill hole diameter = 6.4 mm) was significantly reduced by the ants after 2.5 hrs (median hole diameter = 5.9 mm) (Fig. 1c) (Wilcoxon signed rank test with continuity correction, V = 213.5, $P = 0.002$, $N = 22$; Fig. 3). After 24 hrs ants further reduced the diameter of the opening (median = 4.0 mm) (Figs 1d, 2a, 2b) (Wilcoxon signed rank test with continuity correction, V = 244, $P << 0.001$, $N = 22$), or these holes were completely sealed ($N = 4$).

Using a cut-off value approximately twice the value of natural entrances to internodes, colonies were scored as having worked to repair the damage if the difference between the initial drill hole diameter and the final diameter was > 1.5. With this criterion, slightly more than half the holes were sealed or were only minimally repaired ($N = 14$ of 22; exact binomial test, $P = 0.286$). In at least one case a hole that was categorized as "open" after 24 hrs was sealed after 72 hrs (JET personal observation), but we did not gather temporal data beyond 24 hrs due to pandemic movement restrictions (see Methods). In those trees in which ants did respond, using the above crite-

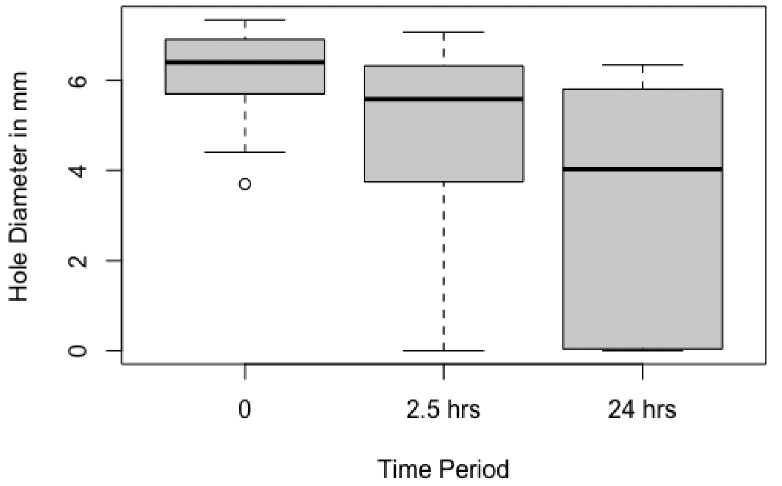

Figure 3. Boxplot of hole diameters 2.5 hours and 24 hours after a hole was initially drilled into a *Cecropia* stem.

rion, there was no significant association with work effort (as measured by a reduction in the diameter of the hole) and the number of internodes from the top of the tree to the drilled internode (Spearman's rank correlation between number of nodes from the top and hole diameter after 24 hours: rho = 0.2252, S = 352.5, P = 0.4387).

Discussion

Azteca alfari ants tend to repair structural damage to their host plants, with variation among colonies. Behavioral personalities of *Azteca* colonies vary considerably (Marting et al. 2018a,b), but we do not know if repair behavior maps to those behavior types. The reasoning behind hole repair behavior could be because a hole in the wall exposes the ants' vulnerable immature stages to external pathogens, predators, or changes in other environmental parameters (e.g., relative humidity). The importance of such protection is evidenced by the fact that *Azteca* foundresses plug the prostomal entrances right after they open a hole to colonize a new *Cecropia* stem (Mayer et al. 2018; see references in Valverde & Hanson 2011; for a video of this behavior by P. Marting [pers. comm.], see https://www.youtube.com/watch?v=fhNJJE5zZOA).

A change in the internal environment probably explains why ants evacuated brood from compromised internodes. Many organisms that build physical shelters for themselves or their offspring will regularly repair them when damaged (Madeiros et al. 2016; Farji-Brener and Tadey 2016; Downing 1992), as do humans (e.g., Divringi et al. 2019). But here we demonstrate that ants will work to repair a living structure, using plant material from inside the stem. Light-colored parenchyma cells are used by foundress queens to seal the entrance hole they make when colonizing a new plant (references in Valverde and Hanson 2011), which may be the light-colored patch material seen in Fig. 1d.

In a symbiosis, repairing the hole hypothetically would allow the plant to conserve energy on repairing wounds, but this benefit may be unlikely because eventually the trees form natural scars around the wounds using their own defenses (Fig. 2c; see Vasyukova et al. 2011). Ant repair behavior may be unlikely to benefit the plant directly, and thus the behavior may differ from microbial symbionts that facilitate wound healing in their hosts, which is beneficial to both (Poutahidis et al. 2013). However, we could not exclude the possibility that ants apply glandular secretions, which in some ant species have known antimicrobial activity (e.g., Fernández-Marín et al. 2015). If so, plants would benefit from the ants' behavior. We cannot explain why approximately half of the colonies repaired the damage and the others did not or did so minimally, but additional sources of uncontrolled variables would include plant age and colony size, and species of *Cecropia*.

Anecdotal observations of holes drilled into upper internodes of very small saplings (DBH < 1 cm) suggest that damage to internodes containing brood is rapidly repaired and closed within 5 hours, but first brood were evacuated (WTW, pers. obs.). Conversely, extensive damage is not repaired: clay balls shot through upper internodes in small stems with thin walls caused extensive damage, leaving frayed entry and exit wounds that were not repaired (AW, WTW pers. obs.). We hypothesize that colonies that did not respond in a short time frame had their broods far up in the tree, such that minor damage to a long-abandoned internode represents minimal risk for them, but we did not sacrifice the trees and ants, nor had access to a hydraulic lift to study colonies in larger trees to test it.

Sling shots wielded by teen-agers are a novel threat to the ants' shelter, but in nature *Bradypus* sloths are frequent visitors to *Cecropia* trees (Leigh 2002), and their sharp toenails sometimes pierce the wood (Peter Marting, personal communication, 2020), as would those of silky anteaters (*Cyclopes*). These openings likely would be smaller than the size of our experimental holes. These results of home-repair behavior in a symbiont reveal a new level of attention by the ants to their host plants. The ants not only behave in ways to minimize damage to their hosts, but when damage does occur, they actively work to fix it, albeit for their own benefit.

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Supplementary material 1

Video S1

Authors: Alex Wcislo, Xavier Graham, Stan Stevens, Johannes Ehoulé Toppe, Lucas Wcislo, William T. Wcislo

Data type: Mp4 file.

Explanation note: Azteca ant repair behavior approximately 15 min after a 6.4 mm hole was drilled into an internode near the terminal bud; recorded with a 20.6 MP Sony Handycam FDR-AXP35 (normal speed). Video edited with OpenShot Video Editor 2.5.1.

Link: https://doi.org/10.3897/jhr.88.75855.suppl1

Supplementary material 2

Video S2

Authors: Alex Wcislo, Xavier Graham, Stan Stevens, Johannes Ehoulé Toppe, Lucas Wcislo, William T. Wcislo

Data type: Mp4 file.

Explanation note: Azteca ant repair behavior approximately 2.5 hr after a 6.4 mm hole was drilled into an internode near the terminal bud; recorded with a 20.6 MP Sony Handycam FDR-AXP35 (playback speed, 4X speed). Video edited with OpenShot Video Editor 2.5.1.

Link: https://doi.org/10.3897/jhr.88.75855.suppl2

Supplementary material 3

Azteca ants repair damage to their Cecropia host plants

Authors: Alex Wcislo, Xavier Graham, Stan Stevens, Johannes Ehoulé Toppe, Lucas Wcislo, William T. Wcislo

Data type: Docx file.

Explanation note: The following statistical tests Statistical tests were done using a package in R4.0.3 and RStudio 1.3.1093 (RStudio Team 2020), to test whether data met assumptions for normality.

Link: https://doi.org/10.3897/jhr.88.75855.suppl3