SCIENTIFIC NOTE

Ants Do Not Traverse the Silk of Adult Female *Nephila clavipes* (Linnaeus) Webs

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Abstract

Many organisms use chemicals to deter enemies. Some spiders can modify the composition of their silk to deter predators from climbing onto their webs. The Malaysian golden orb-weaver *Nephila antipodiana* (Walckenaer) produces silk containing an alkaloid (2-pyrrolidinone) that functions as a defense against ant invasion—ants avoid silk containing this chemical. In the present study, we test the generality of ants' silk avoidance behavior in the field. We introduced three ant species to the orb webs of *Nephila clavipes* (Linnaeus) in the tropical rainforest of La Selva, Costa Rica. We found that predatory army ants (*Eciton burchellii* Westwood) as well as non-predatory leaf-cutting ants (*Atta cephalotes* Linnaeus and *Acromyrmex volcanus* Wheeler) avoided adult *N. clavipes* silk, suggesting that an additional species within genus *Nephila* may possess ant-deterring silk. Our field assay also suggests that silk avoidance behavior is found in multiple ant species.

Introduction

Ants are ecologically abundant and diverse organisms, including many species that are consumed by arthropods as well as many species that pose a predatory risk to other arthropods. Defensive traits successful in deterring ants are thought to be beneficial for potential prey. For example, *Polistes fuscatus* (Fabricius) wasps secrete ant-repellent chemicals on the stem of their nest as a way to prevent ant invasion (Post & Jeanne 1981, Posy *et al* 1984), and sawfly larvae (*Arge* spp.) defend against ant predators via repellent volatile emissions and potentially toxic hemolymph (Petre *et al* 2007). More recently, Zhang *et al* (2012) presented evidence for ant-repelling properties of spider silk, the first and only test to date showing that spider silk may chemically defend against ants.

Ant–spider associations are diverse, including mimicry, parasitism, and predatory specialization (Cushing 2012). Even though ants have been documented as prey for multiple species of spiders, in general, spiders avoid eating ants (Cushing 2012). On the other hand, ants are also predators of spiders. For example, the tropical army ant *Eciton burchellii* (Westwood) hunts for mostly insect and arachnid prey in large swarm raids on the forest floor with some raiding masses that also extend into trees (Schneirla 1955, Rettenmeyer 1963). Wandering spiders (Ctenidae) are the most abundant spider prey type in *E. burchellii* raids (Vieira & Höfer 1994, Gasnier & Höfer 2001, but see Otis *et al* 1986), and other army ants are also known to prey upon web-building spiders (Vieira & Höfer 1994). Potential mechanisms of defense against ants might be beneficial for spiders located in the ants' foraging vicinity. For example, female green lynx spiders, *Peucetia viridans* (Hentz), successfully reduce ant predation on their egg sacs by performing guarding behaviors (Fink 1986, 1987). In another example, the orb-weaving spider *Eustala oblonga* (Chickering) inhabits ant-defended acacia trees, responding to ant attacks by suspending itself on a dragline until ant activity wanes (Garcia & Styrsky 2013). However, relatively few field studies to date have explored possibilities for spider orb web defenses against ants.

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Nephila is a genus of spiders that is widespread across tropical and sub-tropical ecosystems (Kuntner *et al* 2013); these spiders thus have the potential to interact as both predator and prey with a diverse group of ants. Malaysian golden orb-weavers, *Nephila antipodiana* (Walckenaer), defend their webs against ant invaders using a chemical repellent (Zhang *et al* 2012). Adult female *N. antipodiana* spiders produce silk laden with an alkaloid chemical (2-pyrrolidinone) that ants avoid—Zhang *et al* (2012) found that three ant species (*Pheidole angulicollis* Eguchi, *Monomorium pharaonis* Linnaeus, and an unidentified species of *Monomorium*) freely traversed isolated strands of scaffold silk with the deterrent removed, but avoided the silk when experimenters added 2-pyrrolidinone back to the scaffold silk.

To test if ants avoid *Nephila* webs in the field, we conducted an experiment to assess the response of both army ants and non-aggressive ant species to orb silk. We introduced ants to the silken capture spirals of adult and juvenile *Nephila clavipes* Linnaeus, an orb-weaving spider species not previously examined for ant-repelling silk properties. Detailed observations of *N. clavipes* in the field have not shown ants to be a predator (Higgins 1992). We found that ants of all tested species avoided the silk of adult *N. clavipes* spiders.

Material and Methods

Study site and species

All research was conducted from 12 to 18 February 2012 at La Selva Biological Station, Heredia Province, Costa Rica. Experiments were conducted in the Holdridge Arboretum between 0900 and 1600 h. Ants from three species were exposed to spider orb webs, including one army ant species (*E. burchellii*) and two species of leaf-cutting ants (*Atta cephalotes* and *Acromyrmex volcanus*). The field site allowed for convenient experimentation, as the leaf-cutting ant species were found foraging in the juvenile and adult spiders' vicinity. Of the ants in the Arboretum, *Ac. volcanus* had an arboreal nest, which is typical for this species (Hogue 1993), and *At. cephalotes* had an underground nest, evidenced by surface mounds (Stevens 1983, Hogue 1993). Both species of leaf-cutting ants were easily collected in the Arboretum on the forest floor, and for *Ac. volcanus*, at the base of a tree trunk.

We collected 200 *Ac. volcanus* foragers (9–12-mm body lengths) traveling outbound from their nest, trapping ants singly in glass vials (length 7.0 cm, diameter of opening 1.7 cm); 100 ants were collected and tested on each of two days. We similarly collected 100 *At. cephalotes* (5–9 mm) foragers traveling outbound from their nest. *Eciton burchellii*
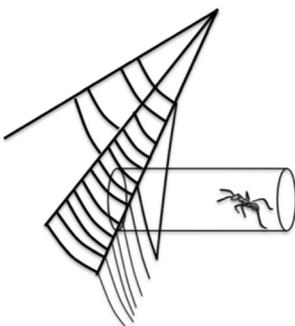
do not maintain fungus gardens, but instead raid the forest floor for arthropod prey (Rettenmeyer 1983, Rettenmeyer *et al* 2011). These ants were not found in the Arboretum; for collecting them, we went out at night (1900 h) to a migrating colony (Rettenmeyer 1983, Hogue 1993) that had been spotted earlier the same day (1600 h) along the intersection of the "STR" and "CEN" trails (Organization for Tropical Studies La Selva GIS Lab 2016). From this colony, we collected 24 *E. burchellii* media workers (7–9 mm). While collecting, E Knowlton disturbed some (at least 100) individuals of this colony and invoked aggressive responses (e.g., the mandibles of multiple major and minor soldiers bit the collecting vials), which made it challenging to collect additional individuals. Major workers were not collected owing to their large mandibles and body size that hindered the ease with which they could fit through the vial opening. In summary, we collected individuals of three species of ants, each from a single colony.

Experiments were conducted with five female *N. clavipes* spiders (Arachnida, Nephilidae): three adults with 7–10-mm carapace lengths, and two juveniles with 3–5-mm carapace lengths, as well as multiple spider webs built by spiders from the genera *Cyclosa*, *Tetragnatha*, and *Micrathena*, including two small abandoned or vacant orb webs built by unidentified spiders ($N \geq 10$). Zhang *et al* (2012) predict that silk of small spiders will not contain the ant-deterring chemical compound, 2-pyrrolidinone, because the silk is sufficiently thin in diameter to prevent ants from traversing it. We included these relatively small, non-*Nephila* spiders as controls in our study to test if silk acted as a physical barrier to ant departure from the vial (see next section). We included small juvenile *N. clavipes* spiders in our study to replicate the approach of Zhang *et al* (2012) since large but not small juvenile *N. antipodiana* silk possesses 2-pyrrolidinone.

Testing ant responses to spider silk

Each individual ant was tested once. For each web trial, the subject's vial was held horizontally next to the capture spiral from one of three types of webs: (1) an adult *Nephila* female, (2) a juvenile *Nephila* female, or (3) a non-*Nephila* sp. (Table 1). We chose to work with silk from the capture spirals rather than scaffold silk because we did not want to risk damage to the webs' support threads; further, not all support threads were within reach as these webs were spun by spiders in natural conditions. Separate samples of ants (50 *Ac. volcanus*, 25 *At. cephalotes*, and 6 *E. burchellii*) were tested with no webs (i.e., an "air control"), wherein the lid from the vial was removed while holding it horizontally (ca. 1 m aboveground) in mid-air (Table 1). This control was meant to test if the vial per se influenced ant behavior or if ants had any motivation to leave the vial. We also tested ant responses to non-*Nephila* orb webs as a silk control. This silk

Table 1 Summary of experimental setup.

Category	Image	Description
Orb web exposures		Held ant within vial up to one of 3 types of orb web silk: (i) adult *N. clavipes*, (ii) juvenile *N. clavipes*, or (iii) non-*Nephila* orb web
Air control exposure		Held ant within vial (lid removed) to open air

control was meant to test if silk acted as a physical barrier preventing ants from departing the vial.

Before each trial, the vial was tapped lightly to place the ant at the vial's far end away from the lid. A trial began once the vial's opening touched the silk, or for the air control, once the lid was off. In each trial, the ant was free to stay in the vial or leave. Responses were recorded as (1) departure from the vial ("out"), (2) initial movement to the opening of the vial with subsequent retreat and no departure ("in"), or (3) no movement. A trial ended once a response was observed or after two minutes, whichever came first.

Statistical analyses

Tests were performed using R 3.3.2 (R Core Team 2016). We used a logistic regression to assess the effect of treatment (exposure to adult *Nephila*, exposure to juvenile *Nephila*, exposure to a non-*Nephila* web, and air control) and species (*Ac. volcanus* [$n = 172$], *At. cephalotes* [$n = 100$], *E. burchellii* [$n = 23$]) on whether or not the ant left the vial. We compared models with and without an interaction between treatment and species using a likelihood ratio test (using the package *lmtest*; Zeileis & Hothorn 2002).

For trials with *At. cephalotes* and *E. burchellii*, only one adult *Nephila* and one juvenile *Nephila* were used for all trials; however, for *Ac. volcanus*, trials conducted on different days exposed ants to different adult and juvenile webs. To assess if the results were influenced by spider identity, we restricted the dataset to *Nephila*-exposed treatments in *Ac. volcanus* and compared a model with a treatment and spider identity effect to a model with only a treatment effect using a likelihood ratio test.

Results

Our air control trials demonstrate that the vial per se did not prevent ants from making a departure. Across all species of ants tested, 94% of ants left the vial during the air control, revealing that there seemed to be motivation to leave the vial. Ants also departed the vial 55% of the time during the silk control trials. This demonstrates that silk per se did not completely prevent ants from making a departure by acting as a physical barrier. *Acromyrmex volcanus* individuals did not move in 14% of their trials; we do not have evidence for treatment or silk type to have affected this ($\chi^2 = 5.43$, df = 3, $P = 0.143$). These *Ac. volcanus* "no movement" trials were excluded from the logistic regression analysis (see the "Statistical analyses" section) because "no movement" ant behavior was difficult to interpret, as neither the movement to the opening of the vial nor subsequent retreat to the end of the vial (which qualifies as an "in") were observed.

Ants appeared to discriminate among silk types (Figs 1 & 2). Models with and without an interaction term between the effects of treatment and ant species did not differ significantly (likelihood ratio test: $\chi^2 = 10.6$, df = 6, $P = 0.10$), so only the model without the interaction was considered further. Across all ant species, ants were significantly more likely to remain within vials when exposed to adult *Nephila* webs than when exposed to juvenile *Nephila* ($\beta \pm SE = 2.3 \pm 0.4$, $z = 5.3$, $P < 0.001$), other non-*Nephila* spiders ($\beta \pm SE = 2.3 \pm 0.4$, $z = 5.2$, $P < 0.001$), or the air ($\beta \pm SE = 4.6 \pm 0.6$, $z = 7.8$, $P < 0.001$). Eighty percent of *At. cephalotes*, 83% of *E. burchellii*, and 68% of *Ac. volcanus* individuals remained within the vial when exposed to adult *N. clavipes* webs. Ants were more likely to leave the vial when exposed to air than when exposed to juvenile *Nephila* ($\beta \pm SE = 2.3 \pm 0.5$, $z = 4.4$, $P < 0.001$) or other spiders ($\beta \pm SE = 2.3 \pm 0.5$, $z = 4.5$,
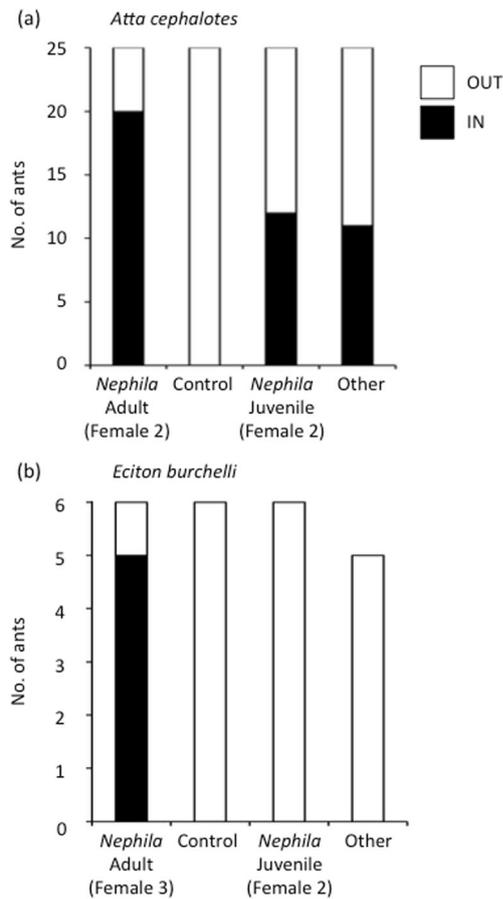
Fig 1 Leaf-cutter ant (*Atta cephalotes*, a) and carnivorous ant (*Eciton burchellii*, b) responses to spider silk of different types (adult: silk from the orb web of an adult *N. clavipes* female; control: an open vial held in mid-air; juvenile: silk from the orb web of a juvenile *N. clavipes* female; other: silk from the orb web of a non-*Nephila* spider). Ants stayed in the vial (IN = black bars) or exited the vial (OUT = white bars). Adult and juvenile *Nephila* spider ID in parentheses below the *x*-axis.
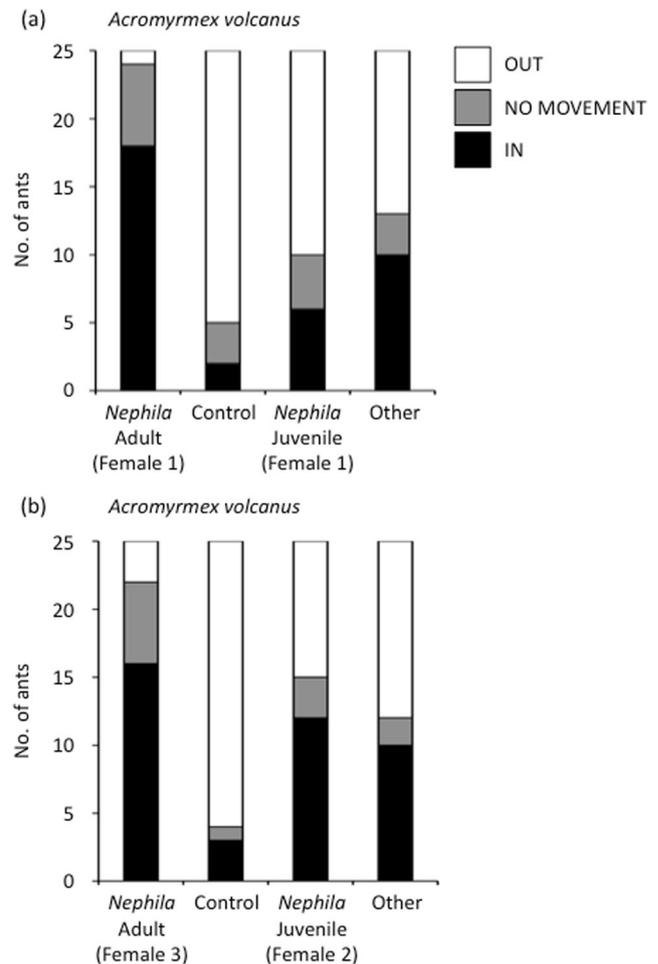

Fig 2 Leaf-cutter ant (*Acromyrmex volcanus*) responses to spider silk of different types for the initial (a) and within-species replicate (b) experiments. Adult: silk from the orb web of an adult *N. clavipes* female; control: an open vial held in mid-air; juvenile: silk from the orb web of a juvenile *N. clavipes* female; other: silk from the orb web of a non-*Nephila* spider. Ants stayed in the vial (black bars), exited the vial (white bars), or did not move (gray bars). Adult and juvenile *Nephila* spider ID in parentheses below the *x*-axis.

$P < 0.001$), but their response to juvenile *Nephila* webs was not distinguishable from their response to other non-*Nephila* spider webs ($\beta \pm SE = 0.0 \pm 0.3$, $z = 0.03$, $P = 0.97$). Behaviorally, ants that left the vial attempted to traverse orb silk or—as was typically the case in the air control trials—climbed on the outside of the vial. Ant responses categorized as "in" meant that ants walked to the tip of the vial, antennated the silk present, and turned around, walking back to the end of the vial.

Across all treatments, *E. burchellii* ants were more likely to leave the vial than either *Ac. volcanus* ($\beta \pm SE = 1.7 \pm 0.7$, $z = 2.7$, $P = 0.008$) or *At. cephalotes* ($\beta \pm SE = 1.5 \pm 0.6$, $z = 2.3$, $P = 0.02$); Fig 1 suggests that this is primarily driven by *E. burchellii* leaving the vial in 100% of trials when exposed to the webs of juvenile *Nephila* and other spiders, but as noted above, the interaction between treatment and ant species was not significant. The two leaf-cutter ant species had similar propensities to leave the vial across treatments ($\beta \pm SE = 0.2 \pm 0.3$, $z = 0.7$, $P = 0.47$).

For *Ac. volcanus*, which were exposed to one of two adult or two juvenile *Nephila*, the identity of the spider to which the ants were exposed did not have a significant effect on their propensity to leave the vial (likelihood ratio test: $\chi^2 = 4.2$, df = 2, $P = 0.12$).

Discussion

An interpretation of our results suggests that predatory army ants (*E. burchellii*) and non-predatory leaf-cutting ants (*At. cephalotes* and *Ac. volcanus*) may discriminate against the orb webs of adult female *N. clavipes* compared to the orb webs of juvenile *N. clavipes* and non-*Nephila* orb-weavers, as well as air controls. Previous work shows that the support strands of congeneric *N. antipodiana* orb webs contain an

ant-deterring alkaloid, 2-pyrrolidinone (Zhang *et al* 2012). The presence of this chemical functions as a defense against ant predation; individuals of three ant species were shown to avoid silk containing that chemical but not silk with the chemical removed (Zhang *et al* 2012). Our study may provide further support that ants avoid spider silk. Here, we present evidence that ants avoid adult *N. clavipes* orb webs. However, we did not test the chemical properties of adult *N. clavipes* orb webs in our study, as has been done with the support threads of adult *N. antipodiana* and *Argiope keyserlingi* (Karsch) webs, showing the presence of alkaloids (Zhang *et al* 2012, Henneken *et al* 2017). Until *N. clavipes* silk is analyzed for ant-deterring compounds, we interpret our results with caution acknowledging that silk mechanical cues may have formed the basis for ant discrimination, as the physical properties of silks may be different. However, if silk as a mechanical cue or barrier would have prevented ants from leaving the vial, then we would have expected a greater percentage of ants to remain in the vial in the presence of non-*Nephila* silk controls and juvenile *N. clavipes* silk than was observed. Consistent with the prediction of Zhang *et al* (2012) that small spiders lack silk with ant-deterring chemicals (because their silk is sufficiently thin to limit access by ants), ant responses to non-*Nephila* webs and juvenile *N. clavipes* webs were explained by chance alone. However, we will interpret this result with caution as well because we only sampled ant responses to two juvenile *N. clavipes* webs. Moreover, since a greater percentage of ants exited the vial for the air control than for any silk treatment, we cannot rule out that silk may have acted as a physical barrier to ants.

From the spiders' perspective, ant web avoidance, especially the high avoidance of adult *N. clavipes* orb webs, may compromise the spiders' ability to acquire non-predatory ants as prey. However, the majority of spider species do not accept ant prey (Foelix 2011, Cushing 2012), in which case ants' avoidance of orb webs may be beneficial to both ants and spiders. Indeed, *N. clavipes* may occasionally prey on alate ants caught in the web but these ants are mostly ignored (Higgins 1987). On the other hand, predatory ants consume arachnid prey (Gillespie & Reimer 1993, Gasnier & Höfer 2001, Wilson 2005, Elżanowski & Czechowski 2015), so why might *E. burchellii* army ants avoid *N. clavipes* orb webs? One possibility is that *Nephila* could employ compounds for which army ants had a pre-existing revulsion, a form of aposematic sensory exploitation through chemistry. Similarly, foraging leaf-cutting ants select against certain leaves based on plant defensive compounds, including alkaloids (Howard 1988, Howard *et al* 1988, Hol & Van Veen 2002), and it is possible that the avoidance of spider predators could be mediated by similar chemical cues. Future work may adopt silk sampling methods (Higgins *et al* 2001, Henneken *et al* 2017) paired with ant behavioral assays

(Zhang *et al* 2012) to explore the mechanisms of web avoidance and silk defense.

Author Contribution   EK designed the project and collected the data; EK and AK analyzed the data and wrote the manuscript.

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