The Dissertation Committee for Zachary Isaac Phillips Certifies that this is the approved version of the following Dissertation:

Dispersal of *Attaphila fungicola*, a Symbiotic Cockroach of Leaf-cutter Ants

Committee:

Larry E. Gilbert, Supervisor

Ulrich G. Mueller, Co-Supervisor

Shalene Jha

Timothy Beach

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by

Zachary Isaac Phillips

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Dedication

To Brackenridge Field Lab, Hornsby Bend, Café Medici and the Austin Film Society.

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Thank you Caroline Farrior for not kicking me out of your office when I entered unannounced with 50 pages of algebra and sketches of a model diagram (see Chapter 3).

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Thank you cockroaches. You are not meddling. That was a lie. You are an inspiration.

Finally, I owe everything to my family and friends. None of you are going to read this dissertation, not even this acknowledgement, but I forgive you.

Abstract

Dispersal of *Attaphila fungicola*, a Symbiotic Cockroach of Leaf-cutter Ants

Zachary Isaac Phillips, PhD
The University of Texas at Austin, 2021

Supervisors: Larry E. Gilbert and Ulrich G. Mueller

Animal dispersal between habitats is difficult to observe from beginning to end. This is especially true of tiny, cryptic animals resistant to tracking methods. *Attaphila fungicola* is a miniature cockroach that lives in the deep, subterranean nests of leaf-cutter ants, a symbiont of the leaf-cutters and their mutualist fungal gardens. The only conspicuous glimpse we get of *A. fungicola* dispersal is at the beginning of their journey, during the nuptial flight preparations of leaf-cutter colonies. During these preparations, *A. fungicola* hitchhikes on the colony's female alates (winged queens), which, if successful, will mate mid-air with male alates, land and begin new leaf-cutter colonies as foundresses (workerless queens). Hitchhiking on female alates has long been interpreted as a roach behavior facilitating dispersal to incipient colonies of foundresses; however, incipient colonies likely represent much lower quality habitats than larger established colonies, and roaches may benefit by avoiding the former during dispersal in favor of arriving at the latter. I explore this possibility under a host-symbiont framework, describing *A. fungicola*'s dispersal to incipient host colonies as "vertical transmission," and its dispersal between larger established colonies as "horizontal transmission." By considering variation in host quality between incipient and established colonies, and by using surveys, lab and field experiments and a mathematical model, I find evidence that *A. fungicola* roaches are primarily horizontally transmitted between established colonies and may use a mode of roach dispersal that entails two hitchhiking steps – first on female alates emigrating from *upstream* host colonies, then on foragers returning to *downstream* host colonies ("The Texas Two-step"). These findings have broad implications for predicting the dispersal/transmission of organisms that co-disperse (e.g., hitchhike) with their host's propagules (e.g., female alates, plant seeds) and validate the importance of incorporating colony development into studies of host-symbiont dynamics.

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INTRODUCTION

Many organisms use colonies of ants, bees, wasps and termites as sources of food, shelter and protection. Those that form close, durable relationships with a colony can be considered colony "symbionts" (Combes 2001). Colony symbionts harm, benefit, or have a negligible effect on their host colony's survival and reproduction, acting as parasites, mutualists or commensals, respectively (Schmid-Hempel 1998; Combes 2001). They represent a broad range of taxa (Schmid-Hempel 1998; Kronauer and Pierce 2011), and range correspondingly in size from viruses to trees (Gaume et al. 1998; Allen et al. 2011).

An extensive literature exists on the association between colonies and their symbionts, with researchers approaching the subject from various behavioral, ecological, evolutionary and applied perspectives (Schmid-Hempel 1998; Cremer et al. 2007; Hojo et al. 2009; Kronauer and Pierce 2011; Campbell et al. 2013; Ivens et al. 2016; Powell et al. 2016; Mueller et al. 2017; LeBrun et al. 2018; Adams et al. 2020); however, under all research lenses the influence of colony development on symbiont biology has largely been neglected. Like individuals, colonies undergo development (Wilson 1985; Tschinkel 1993; Smith et al. 2016). At both the scale of the individual host (e.g., solitary bee) and colonial host (e.g., bee colony), development can alter the host's phenotype and thus the "host environment" (Rynkiewicz et al. 2015) that symbionts potentially encounter and inhabit. Colony development can transform a single individual (i.e., the new queen) without workers into a complex superorganism with millions of workers (Wilson 1985; Tschinkel 1993; Hölldobler and Wilson 2009; Smith et al. 2016), dramatically changing the colony's size, resources, defenses, foraging behavior, nest architecture, interaction networks, and other qualities that can affect symbiont fitness (Wilson 1985; Schmid-Hempel 1998; Combes 2001; Cremer et al. 2007; Hughes et al. 2008; Smith et al. 2016;

Adams et al. 2020). As a consequence, colony development is likely an important but understudied factor shaping symbiont ecology and evolution (Yang 2007; Moret and Schmid-Hempel 2009; Manfredini et al. 2016).

The development of individual insects (i.e., individual ontogeny) is known to affect the transmission of their symbionts (Briggs and Godfray 1995; Hammer et al. 2014), and the development of insect societies (i.e., colony ontogeny) may similarly affect the between-colony transmission of their symbionts. Transmission is a central feature of host-symbiont dynamics (Fine 1975; May and Anderson 1987; Lipsitch et al. 1996; Genkai-Kato and Yamamura 1999) and represents a particular form of dispersal – the dispersal of symbionts between hosts (note, I use “dispersal” and “between-colony transmission” interchangeably) (Antonovics et al. 2017). In this dissertation, I explore how colony development affects between-colony transmission of symbionts. Specifically, I explore how early stages in the development of a Texas leaf-cutter ant colony (*Atta texana* Buckley) affect the dispersal of a symbiotic cockroach, *Attaphila fungicola* Wheeler. By adopting a colony-developmental perspective, and by incorporating early stages of colony development into conceptual, empirical and mathematical frameworks, I discovered a novel mode of *A. fungicola* dispersal and demonstrate that horizontal transmission is the roach’s dominant route of transmission, results deviating from traditional assumptions. These findings improve our understanding of host-symbiont dynamics and the evolution of dispersal, and have implications for a wide range of symbionts (elaborated in the Discussion sections of Chapters 2 and 3).

CENTRAL HYPOTHESES AND CHAPTER SUMMARIES

Routes of symbiont transmission can be divided into two general categories: horizontal transmission and vertical transmission (Antonovics et al. 2017). Vertical transmission refers to routes that carry symbionts from parent to offspring hosts (Fine 1975; May and Anderson 1987; Lipsitch et al. 1996) and horizontal transmission describes every other route of transmission between hosts. In this dissertation, vertical transmission refers primarily to transmission from parent colony to incipient offspring colony (Fries and Camazine 2001), and horizontal transmission refers primarily to transmission between established colonies (i.e., ergonomic and mature colonies: post-incipient stages of colony development) (Oster and Wilson 1978).

During leaf-cutter colony nuptial flights, the miniature cockroach *A. fungicola* hitchhikes on female alates (winged “queens”) that mate mid-air, land and begin new colonies as foundresses (workerless “queens”). Traditionally, hitchhiking on female alates has been interpreted as a behavior that facilitates roach vertical transmission from leaf-cutter parent colony to incipient daughter colony (i.e., the foundress stage of colony development), and vertical transmission has been considered the dominant route of roach transmission (Moser 1967*b*). I describe these assumptions as the following two hypotheses, respectively:

(1a) *Attaphila fungicola* only uses female alates for vertical transmission

(2a) Vertical transmission is the roach’s dominant route of transmission

In this dissertation, I test the following alternative hypotheses:

(1b) *Attaphila fungicola* can use female alates as vectors for horizontal transmission

(2b) Horizontal transmission is the roach’s dominant route of transmission

Chapter 1 includes my natural-history account of field observations that led me to doubt traditional hypotheses (1a) and (2a) and to formulate and design studies to test hypotheses (1b) and (2b). I describe observations of *Attaphila fungicola* behavior in both artificial nests of queens with workers (established colonies) and artificial nests of foundresses without workers (incipient colonies), and report experimental results that suggest incipient colonies are much lower quality hosts for *A. fungicola* than established colonies, a premise of hypotheses (1b) and (2b).

In Chapter 2, I describe behavioral experiments in the field testing whether *A. fungicola* can use female alates as vectors for horizontal transmission (Hypothesis 1b). In particular, I test if *A. fungicola* abandons female alates/foundresses as they search for and begin excavating new nests, and if *A. fungicola* can use foraging lines of established colonies to reach and enter (i.e., "infect") established colonies. The results indicate *A. fungicola* can use female alates for horizontal transmission between established colonies, and I discover that roaches can hitchhike on leaves carried by leaf-cutter foragers to reach and enter the nests of established colonies. I identify "propagule-mediated transmission" as a mode of transmission in which symbionts use their host's broadcast propagules (e.g., female alates, plant seeds) as vectors to reach older conspecific hosts, and suggest this may be a common but underreported phenomenon due to assumptions of vertical transmission.

In Chapter 3, I describe a mathematical model and corresponding experiment testing if vertical (Hypothesis 2a) or horizontal transmission (Hypothesis 2b) are the dominant route of *A. fungicola* transmission. The results indicate that *A. fungicola* is not well adapted to persist through the foundress stage of colony development, and that horizontal transmission is predicted to be the dominant route of *A. fungicola* transmission. I discuss potential constraints on the evolution of vertical transmission for roaches and other symbionts.

In Chapter 4, I describe myrmecophilic (ant-associated) behaviors of an invasive ant-mimicking spider, *Falconina gracilis* Keyserling. I find that *F. gracilis* displays a novel mode of ant predation, usurps the new nests of Carpenter ant foundresses (*Camponotus sansabeanus*), and exploits established colonies of Texas leaf-cutter ants. These observations suggest that *F. gracilis* may affect ant communities by disproportionately killing the foundresses of particular ant species.

CHAPTER 1

Preliminary observations and experiments indicating incipient colonies are low quality hosts

Abstract:[1]

Attaphila fungicola roaches were collected during *A. texana* nuptial flight preparations and placed in mini-nests assembled with multiple chamber types (fungal, queenright fungal, empty, arena). In these mini-nests, which approximate small established colonies of leaf-cutter ants, I observed and documented *A. fungicola* roach behaviors and distribution. Roaches did not avoid queenright chambers and were not attacked more frequently by workers in queenright chambers, deviating from my expectations. *A. fungicola* were also placed in artificial incipient nests, each with an *A. texana* foundress. The survivorship of *A. fungicola* in these incipient nests was measured and compared with *A. fungicola* survival in a non-natal established fungal garden tended by *A. texana* workers. After 13 days, 100% of *A. fungicola* had died in incipient nests with foundresses, while 100% of *A. fungicola* remained alive in the fungal garden tended by *A. texana* workers. Additionally, *A. fungicola* were placed in a foraging chamber of a laboratory colony to determine if, once in the nest, roaches could navigate to the fungal garden chamber; 100% of *A. fungicola* entered the fungal garden chamber. Alate preference (female vs. male) of *A. fungicola* was tested by placing roaches separately in

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[1] Part of this chapter and supplementary videos are published in Phillips, Z. I., M. M. Zhang, and U. G. Mueller. 2017. Dispersal of *Attaphila fungicola*, a symbiotic cockroach of leaf-cutter ants. Insectes Sociaux 64:277–284. Z.I. Phillips designed the experiments, collected and analyzed data and wrote the paper, M.M. Zhang helped analyze data, and U.G. Mueller contributed observations and helped write the paper.

containers with one *A. texana* female alate and one male alate; after 2 h, 71% of *A. fungicola* were attached to female alates and 0% to male alates. These accumulated observations and results indicate incipient colonies are low quality hosts compared to established colonies, and corroborate previous field observations of roach preference for hitchhiking on female alates. Since female alates ostensibly facilitate roach encounters with incipient colonies (because female alates *become* incipient colonies), the following question arises: Even though hitchhiking on female alates facilitates roach encounters with incipient colonies (vertical transmission), can roaches instead hitchhike on female alates as a way to reach higher quality established colonies (horizontal transmission)?

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INTRODUCTION

Studies on an animal behavior often begin with preliminary observations and experiments, a period that allows researchers to become familiar with their subjects and form hypotheses (Bateson and Martin 2021). This chapter includes observations and pilot studies of *Attaphila fungicola* behavior in both artificial nests of queens with workers (established colonies; **Figure 1.1** and **Table 1.1**) and artificial nests of foundresses without workers (incipient colonies; **Figure 1.2** and **Table 1.2**). These "within-colony" observations of *A. fungicola* underlie the development of my two main hypotheses regarding roach between-colony transmission (hypotheses 1b and 2b in Introduction).

To observe behaviors and interactions of *A. fungicola* in established colonies, and to test hypotheses regarding roach behavior and distribution, I assembled leaf-cutter mini-nests in lab with multiple nest chamber types (see **Figure 1.1**). These mini-nests were used to test the following hypotheses: (1) *A. fungicola* avoid queenright fungal garden chambers (fungal garden chambers with the queen present), and (2) *A. fungicola* are more susceptible to worker attacks in queenright fungal garden chambers than fungal garden chambers without queens. The rationale for these hypotheses is that workers fiercely protect their queen (Franks and Sendova-Franks 2000; Sousa-Souto and Souza 2006; Denis et al. 2008), and as a consequence roaches may be more susceptible to worker attacks in the presence of the queen and thus avoid her proximity.

Similarly, to observe behaviors of *A. fungicola* in incipient colonies, and to test whether individual roaches are compatible with incipient colonies and can persist through the foundress stage of colony development, I assembled artificial foundress chambers. Additionally, I conducted choice experiments to test if *A. fungicola* prefers to attach to either *A. texana* female or male alates, and observed whether roaches introduced into a non-fungal garden chamber of a large leaf-cutter colony (the display colony at Brackenridge Field Lab) tend to move to the colony's fungal garden chamber.

METHODS

For all observations and experiments other than those conducted for the multi-chamber nests, *Atta texana* alates were collected from a mature colony at Brackenridge Field Laboratory on 25 April 2015, 7 May 2015, 12 May 2016, and 15 May 2016 between 3 AM and 6 AM, within 3 h before mating flights starting at approximately 5:50 AM (Central summer time). Alates were collected either individually in sterile 5-dram (18.5 mL) polystyrene vials (Thorton Plastics, Salt Lake City) or en masse in 1-gallon sealable plastic bags and transported to a laboratory at the University of Texas at Austin, where alates were kept at room temperature until further study. Female alates (n = 248 on 25 April 2015; n = 1390 on 7 May 2015; n = 514 on 12 May 2016; n = 719 on 15 May 2016) and males (n = 186 on 7 May 2015) were captured in separate containers, and both sexes were examined for *A. fungicola* riding on the alates. For the *A. fungicola* survivorship and alate preference experiments, each *A. fungicola* collected into a vial remained with its host alate, whereas any *A. fungicola* that became dislodged from alates in the plastic bags were moved into a 5-dram vial and paired there with a randomly chosen female alate. The *A. fungicola* and associated host alates remained confined in their respective 5-dram vials for up to 24 h, until experiments were initiated in the laboratory.

Attaphila fungicola used in multi-chamber nest observations were collected during the early morning mating flight preparations of two leaf-cutter colonies at Brackenridge Field Laboratory on 21 May, 2017.

Multi-nest chamber behaviors and distribution of *A. fungicola*

Four mini-nests were constructed using *A. texana* laboratory colonies immediately after *A. fungicola* capture (See **Figure 1.1**). Each mini-nest consisted of the three chambers (7.5 cm x 7.5 cm x 3 cm height plastic container) arranged in a triangle within a larger arena (30 cm X 18 cm X 10 cm height). The experimental treatments are the following: 1) *Queenright Chamber* (QR), containing the queen, fungal garden, and accompanying workers and brood transferred with the fungal garden, 2) *Fungal Garden Chamber* (FG), containing all the previously described colony elements except the queen, and (3) *Empty Chamber*, containing none of the previously described colony elements. The chambers were filled at the base with approximately 56 mL of moistened plaster; the QR and FG chambers each received approximately 112 mL of fungal garden (an estimate based on volume of the garden filled in the container). A single opening in the side of each chamber allowed movement of workers, *A. fungicola* roaches, and the queeninto the arena and among the other chambers. Experiments and observations were initiated a day after mini-nest construction and *A. fungicola* capture. 15 *A. fungicola* were introduced into each mini-nest with introductions of individual *Attaphila* spaced by approximately 10-minute intervals. Experiments of mini-nests were observed under normal lab light, which may have influenced queen-worker-*A. fungicola* interactions**.**

Observations of within-nest distribution and behavior of *A. fungicola* were conducted for six days following *A. fungicola* capture. To assess *A. fungicola* distribution within each mini-nest, five counts every 24 hours were conducted. For each count, the observer scanned each chamber and arena for 60 seconds for living *A. fungicola*. *A. fungicola* cadavers were searched for separately and unseen individuals (those living or dead individuals of the original 15 not observed) were noted. To collect data on the frequency of behaviors among nest chambers within each mini-nest (see **Table 1.1** for list and description of behaviors scored), focal individuals were randomly selected five times each day in each chamber and arena and observed for 180 seconds at a time. Due to my inability to count workers hidden in the garden, worker density was recorded in the empty chamber, but not in the fungal garden chambers.

I used a Pearson's chi-square test to assess the null hypothesis of the uniformity in the distribution of *A. fungicola* occupation among chamber types (excluding the arena) and two-sample Z tests for proportions to determine whether there was a significant difference between the number of roaches observed in FG chambers and the QR chambers, and between the QR chambers and Empty chambers. I also used a Pearson's chi-square to assess the null hypothesis of the uniformity in the distribution of behaviors among nest chamber types, and to test the difference in the frequency of particular behaviors in pairwise comparisons of nest chamber types. For this last analysis, five hypotheses in total are tested and therefore the statistical level is adjusted to $\alpha/5=.01$ according to the Bonferroni correction (i.e., to control for the multiple testing problem).

Bonferroni corrections are typically considered highly conservative in adjusting the crtiterion for Type 1 error under multiple comparisons. However, the number of hypotheses considered here is fairly low, so it should not represent a severe diminishment of statistical power.

Roach movement after introduction into non-fungal garden chamber of large lab colony

Within 1 h of capture, *A. fungicola* were separated into two groups: *A. fungicola* remaining attached to their original vectoring alates (Attached treatment, n = 12) and *A. fungicola* removed from vectoring alates and placed alone (Solitary treatment, n = 9). After 1 h of acclimation, individuals were separately introduced from each treatment into an internal, non-fungal garden chamber of a mature laboratory colony (the display colony at BFL) of *A. texana*; this chamber was connected to a fungal garden chamber on one side and to a foraging chamber on the other side. During a 3-h observational period, I recorded whether or not individual *A. fungicola* from each treatment remained in the chamber in which they were introduced, or dispersed into the fungal garden chamber or foraging chamber.

Attaphila fungicola survivorship in incipient colony vs. established garden

Atta texana foundresses (n = 52) searching for nest sites were collected from a parking lot in Cedar Park, TX (30Åã19′5.86"N 97Åã39′34.72″W) by N. Jones on 7 May 2015 and transported to the laboratory. Each foundress was transferred into an artificial brood chamber constructed from a 5-dram vial filled 75% with moistened dental plaster. In addition, an artificial fungal garden chamber was assembled by transferring approximately 207 cm. of fungal garden, including the workers tending it, from a lab colony of *A. texana* into a round translucent plastic container (4 cm h ~ 11 cm diameter). A digital microscope camera (Dino-Lite AM3111T) was used to film interactions between *A. fungicola* and *A. texana* workers in the artificial garden. Within 48 h of alate capture, *A. fungicola* were randomly assigned to different treatments by transferring 26 *A. fungicola* into separate artificial brood chambers, each housing an *A. texana* foundress captured after mating flights (Queen treatment), 26 *A. fungicola* into separate artificial brood chambers without *A. texana* (Isolation treatment, Control), and 23 *A. fungicola* together into the artificial fungal garden chamber (Fungal Garden treatment). The number of *A. fungicola* placed in the Fungal Garden treatment was based approximately on W. M. Wheeler's excavation of an established *A. texana* nest in Austin, TX, in which "more than seventy specimens [were] taken from three of the large gardens" (i.e., an average of >23 A. fungicola per garden) (Wheeler 1900, p. 856). All replicates were kept at room temperature (22–24 C). Humidity in nest containers was always near 100% because of the moistened plaster. *A. fungicola* and *A. texana* queen (foundress) mortality were recorded every 24 h for 17 days. Observations could not be conducted blind, because the key treatment (presence/absence of queen) was obvious to the experimenter. On the final day of the experiment (Day 17), the fungal garden was dissected to count surviving *A. fungicola* in the Fungal Garden treatment, and the presence or absence of incipient fungal gardens in brood chambers of the Queen treatment was recorded.

Kaplan–Meier estimates (Kaplan and Meier 1958) of the survival function *for A. fungicola* and *A. texana* queens were generated using the statistical package R. From the Kaplan–Meier estimates, Greenwood's formula (Greenwood 1926) was used to calculate the confidence interval around the median death time. A log-rank test was used to compare the three *A. fungicola* survivorship treatments and a pairwise log-rank test to compare differences between pairs of treatments. For the three pairwise tests, a Bonferroni adjusted significance level of 0.05/3 = 0.0167 was used.

Attaphila fungicola alate choice experiment

Pairs of male and female *A. texana* alates (n = 14 pairs) captured on 25 April 2015 were each placed into a container, and a single *A. fungicola* was placed in the center separated from the alates by a barrier (inverted Petri dish). After 1 h of habituation, the barrier was removed to release the *A. fungicola* into the container with the male and female alate. After 2 h, each *A. fungicola* was recorded as either attached to the female alate, attached to the male alate, or neither (i.e., resting or moving on the container substrate). A Pearson's Chi-squared test was used to evaluate if *A. fungicola* display a bias in attaching to female or male alates.

RESULTS

During 2015-2016 collections, all female alates carrying *A. fungicola* captured directly into vials (n = 12) hosted a single cockroach. Assuming female alates captured in sealable plastic bags also carried at most one *A. fungicola* per individual, approximately 6% of all captured female alates hosted *A. fungicola* on each of the collecting dates in 2015 (n = 14 *A. fungicola* on 25 April 2015, n = 82 *A. fungicola* on 7 May 2015) and 5% and 7%, respectively, on the collecting dates in 2016 (n = 25 A. fungicola on 12 May 2016, n = 50 *A. fungicola* on 15 May 2016). The *A. texana* colony apparently migrated approximately 100 m between 2015 and 2016, and as a consequence these collections were made at two different nest sites. No *A. fungicola* were observed on male alates (n = 186 males examined individually). Only one *A. fungicola* collected was a mature male A. *fungicola* captured on 7 May 2015, easily identifiable by wings, which females lack. This is the first record of a mature male *A. fungicola* collected during a mating flight of *A. texana*, although other males have been collected in gardens during nest excavations (Wheeler 1900; Waller and Moser 1990). In 2017, 171 *A. fungicola* were collected from 2687 female alates.

Within-nest behavior and distribution of *A. fungicola* (Mini-nest experiment)

Among the three chamber types, there is no evidence that the distribution of *A. fungicola* deviates from a uniform distribution (Pearson's chi-square, p = 0.42, χ^2 = 1.70, df = 2). There is also no difference between the number of *Attaphila* that occupy the Fungal Garden chamber and the Queenright chamber (Z test, p = 0.60, Z = 0.522) nor between the number occupying the Queenright chamber and the Empty chamber (Z test, p = 0.18, Z = 1.34).

For descriptions and frequencies of observed *A. fungicola* behaviors among chamber types, see **Table 1.1**. The behaviors and interactions of *A. fungicola* expressed among nest chamber types shows no evidence that they are uniform (Pearson's chi-square test, p < 0.001, χ^2 = 568.84, df = 18). In particular, *A. fungicola* is sedentary more frequently in the Empty chamber than the chamber with the next highest count of "rest" observations (FG chamber), moves more frequently in the arena than the chamber with the next highest count of "move" observations (Empty chamber), and antennates with workers less frequently in the empty chamber than in the chamber (i.e., Arena) with the next lowest number of "antennation" observations (two-sample Z tests for proportions, Z = 8.611, Z = 9.98, Z = 4.70, respectively all p-values less than .001). There is no difference in observed worker attacks or grazing events between the FG chamber and the QR chamber (two-sample Z tests for proportions, p = 0.03, Z = 2.17 and p =0.08, Z = 1.72 respectively, with corrected alpha value of 0.007).

In the arena, *A. fungicola* were observed in struggles to right themselves from their dorsum ("Flip" behavior in **Table 1.1**), a behavior not observed in any other nest area. *A. fungicola* only engaged in prolonged (>10 seconds) non-aggressive interactions with *A. texana* workers in the QR and FG chambers. During one period of apparent worker agitation, three *A. fungicola* were observed attached to the queen as she exited into the arena and then returned to her nest chamber. Queens were never observed acting biting or self-grooming off attached *A. fungicola*, or relocating into new fungal garden chambers. Observations of two of the mini-nests were discontinued after four days as workers had moved a significant amount of fungal garden into their respective Empty chambers overnight. *A. fungicola* were observed exiting the Queenright chamber four times, the fungal garden chamber twice, and the empty chamber once. In all mini-nests, refuse piles were formed by workers in the arena, and there were no observations of prolonged interactions of *A. fungicola* with the refuse. In the empty chamber, there were never more than 5 workers at a given time. Average number of worker attacks/chamber were not measured, as worker densities were not recorded in FG or QR chambers.

Observed roach behaviors are summarized in **Table 1.1** below, describing the frequency of different roach behaviors and interactions with ants and fungal garden in four types of artificial nest chambers. Roaches were only observed flipping onto their backs (n = 6) in the open Arena chamber, a position that exposed roaches to repeated ant attacks.

Roach movement after introduction into non-fungal garden chamber

100% of *A. fungicola* from both treatments (12/12 Attached, 9/9 Solitary) entered the fungal garden chamber (**Figure 1.4**) from the adjacent chamber (**Figure 1.3**) to which they were introduced. The condition of being attached or unattached to a vectoring female alate had no effect on the outcome of the navigation of *A. fungicola* from an ostensibly unsuitable internal nest chamber to a favorable fungal garden chamber. Upon introduction, the alates were attacked by the lab colony's workers, while the *A. fungicola* appeared to be unharmed

and eventually detached from the alates and moved to the fungal garden chamber. The alates were either killed or moved into the fungal garden chamber (without *A. fungicola* attached), in which case their fate went unobserved.

Attaphila fungicola and foundress survivorship

Table 1.2 and **Figure 1.2** summarize *A. fungicola* mortality in the survivorship assay. Survivorship was significantly different between Queen, Fungal Garden, and Isolation treatments ($p < 0.0001$, log-rank test of survivorship distributions) (Bland and Altman 2004). Pairwise log-rank tests yielded significantly different survival distributions of *A. fungicola* in the Fungal Garden treatment compared to the Queen treatment ($p = 4.62e{-13}$). *A. fungicola* in the Queen treatment also lived longer than in the control, with median survival durations of 5.5 and 3.0 days, respectively ($p = 0.003$). The cause of mortality for most *A. fungicola* was not clear. In the Queen treatment, two individual *A. fungicola* were grasped and crushed to death by foundresses in their respective brood chambers, suggesting that foundress aggression may have been a factor of mortality; however, most roaches appeared to die from non-violent factors such as dessication. In the Queen treatment, the log-rank test showed that there was no significant difference in survivorship between *A. texana* foundresses with *A. fungicola* absent or present in their brood chambers ($p = 0.913$) (**Fig. 1.2**).

Attaphila fungicola alate choice experiment

In the preference experiment, *A. fungicola* were more likely to attach to a female alate ($p < 0.001$, Pearson's Chi-squared test). 10/14 *A. fungicola* attached to a female alate, 0/14 *A. fungicola* attached to a male alate, and 4/14 attached to no alate.

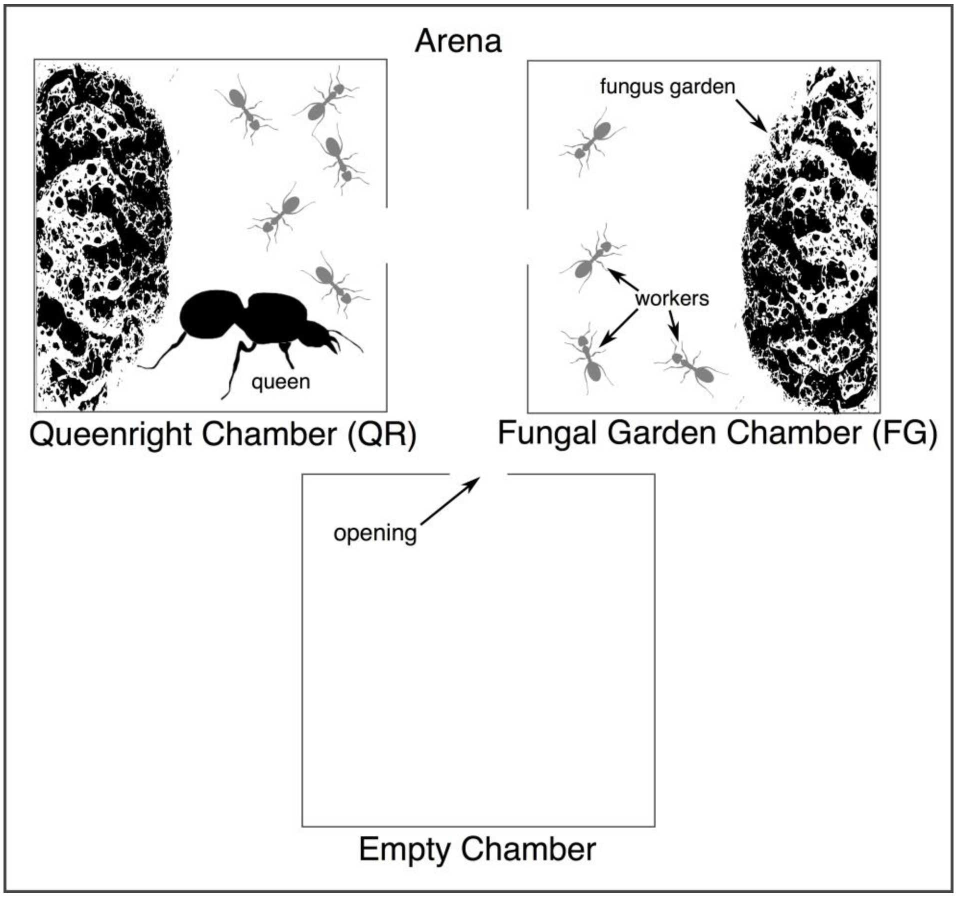

Figure 1.1: Diagram of multi-chamber nest. The distribution of *Attaphila* in different chambers was tracked, and their behaviors and interactions with ants and fungus were recorded.

Table 1.1: *Attaphila fungicola* behaviors in different nest chambers

Key: Attacked = open-mandible attacks by ants on *Attaphila*, GRZ = foraging on fungal garden, PRL = Non-aggressive prolonged interaction with worker (>10s), RES = "Resting," alive but no movement, MOV = continuous movement across entire length of chamber or arena uninterrupted by worker interaction, Flip = Flipping on dorsum and remaining in this position >5 seconds or until worker attack, Antennation = non-aggressive antennal contact with worker

CHAMBERS	Attacked	GRZ	PRL	RES	MOV	Flip	Antennation
Queenright	101	26	9	5	1	0	50
Fungal Garden	77	17	9	11	0	0	52
Empty	3	0	0	68	1	0	4
Arena	60	0	0	1	46	6	31

Table 1.2: Kaplan-Meier Estimates of Survivorship of *Attaphila fungicola*

Table 1.2 Kaplan-Meier Estimates of Survivorship of *Attaphila fungicola*

Treatment	**Number of Experimental Replicates (N)**	**Number of *A. fungicola* per Replicate**	**Total Deaths (after 17 days)**	**Median Death Time (Days)**	**Lower 95% C.I. Death Time (Days)**	**Upper 95% C.I. Death Time (Days)**
Fungal Garden	1	23	1	NA	NA	NA
Queen	26	1	26	5.5	4	8
Isolation	26	1	26	3.0	2	4

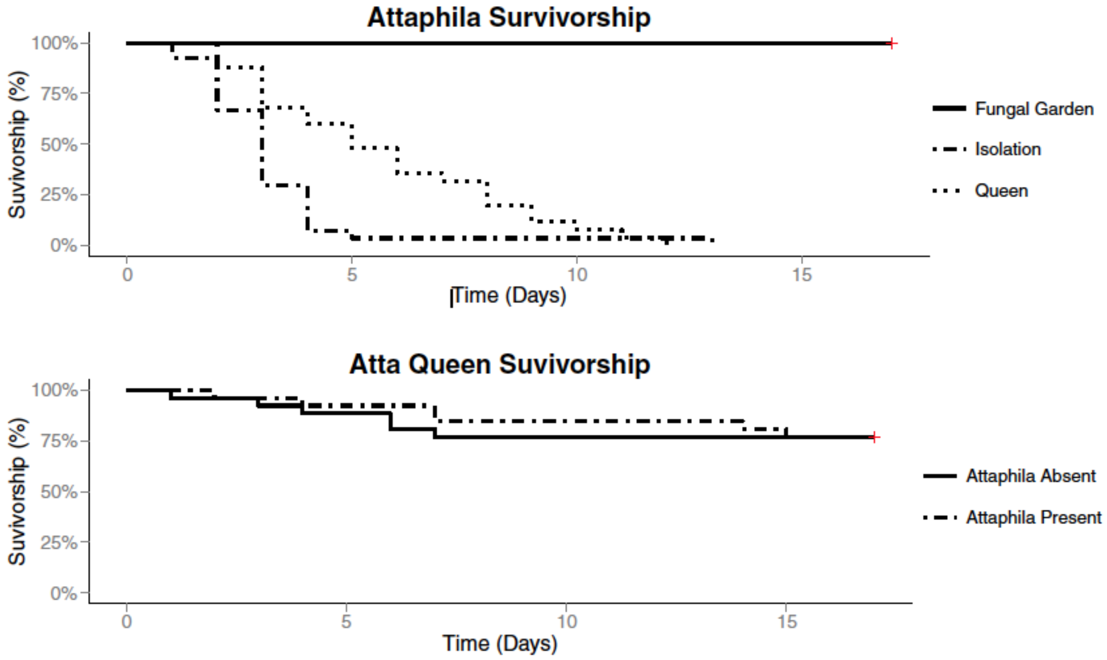

Figure 1.2: Survivorship curves of *A. fungicola* and *A. texana* foundresses

Figure 1.3: *A. fungicola* roach attached to female alate introduced into display colony maintained at Brackenridge Field Lab.

Figure 1.4: *A. fungicola* roaches (center and bottom center) in fungal garden chamber of display colony maintained at Brackenridge Field Lab.

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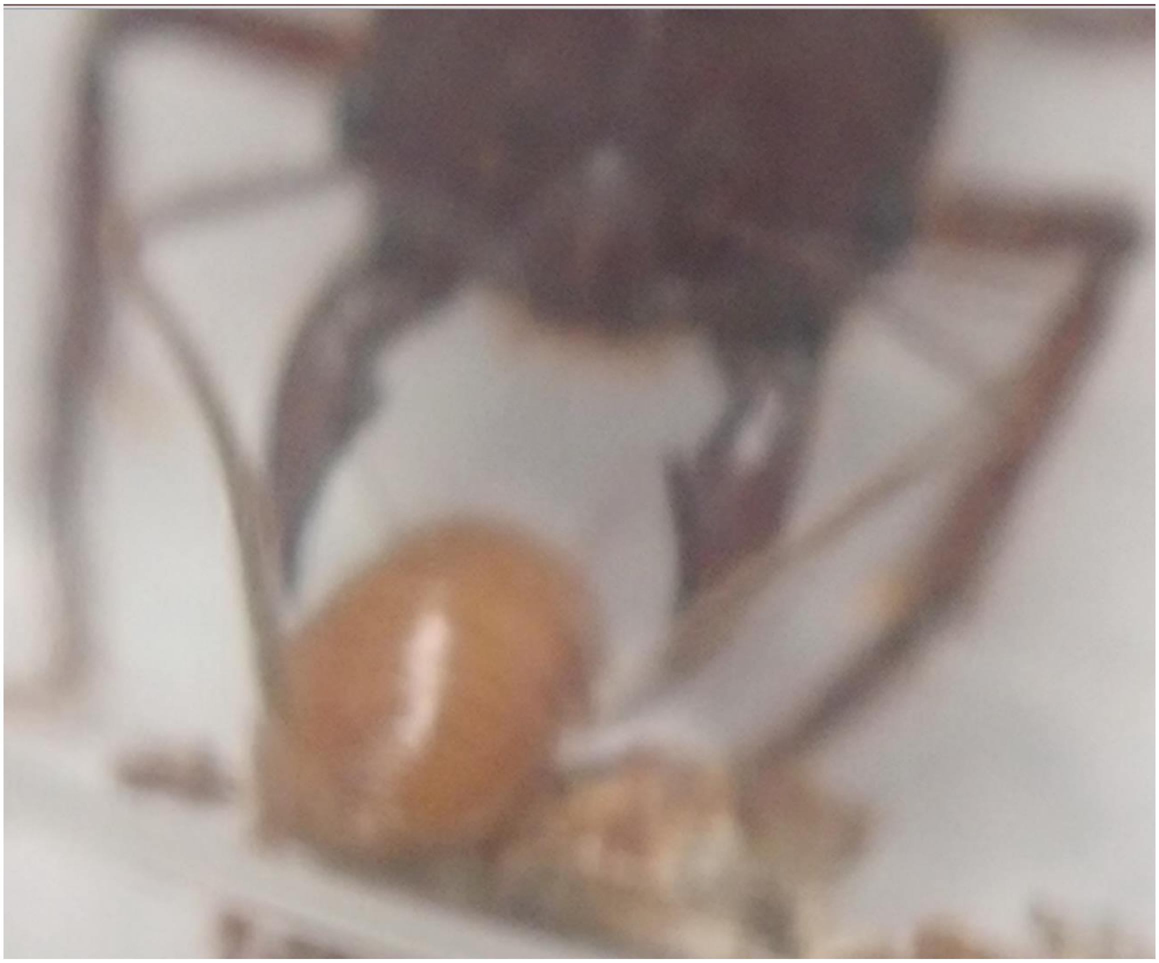

Figure 1.5: The moment before a foundress attacks a *A. fungicola* roach in incipient colony.

DISCUSSION

Collectively, the results suggest *A. fungicola* are more likely to survive in established colonies than in incipient colonies, and that the abundant fungal gardens of established colonies support roaches better than the meager gardens of incipient colonies.

In addition to being a source of food (Wheeler 1900; Nehring et al. 2016), the fungal garden appears to be an important structural resource for roaches and a key mediator of roach-ant interactions. In nest chambers with fungal garden (i.e., queenright and fungal garden chambers), *A. fungicola* were able to maintain agility and balance while avoiding persistently aggressive workers (note, many roach-worker interactions are non-antagonistic as well); in arenas, *A. fungicola* were highly mobile but especially vulnerable to flipping onto their dorsum, a position in which they were invariably attacked; and in empty chambers, *A. fungicola* tended to remain sedentary and attached to the chamber ceiling, rarely interacting with workers or suffering attacks. Thus, *A. fungicola* appear to be able to avoid worker attacks by weaving through the fungal garden matrix and by occupying areas with low worker traffic, whereas they appear especially vulnerable to attacks when encountering workers regularly in areas without fungal garden.

The roaches' use of fungal garden to evade workers is consistent with recent studies that show evasive behaviors rather than just chemical mimicry are important for symbiont integration into colonies (Komatsu et al. 2013; Parmentier et al. 2015, 2017, 2018). In particular, these observations are consistent with studies demonstrating that leaf-cutter colony symbionts such as the social parasite *Acromyrmex insinuator* (a parasitic queen of leaf-cutter colonies) and a species of *Attaphila* can use their host colony's fungal gardens to avoid worker attacks (Nehring et al. 2015, 2016). Fungal gardens could also extend protection to symbionts in close proximity to workers defending the queen, a possible explanation for why there was no apparent effect of the queenright chamber on *A. fungicola* within-nest distribution and frequency of worker attacks. In other words, the structural and/or chemical effects of the fungal garden on roach-worker interactions could overpower possible queen effects on roach-worker interactions.

Incipient colonies have no workers, a colony's main branch of defense against intruders (Cremer and Sixt 2009). Without workers incipient colonies might seem "toothless" against intruders, rendering gardens an unnecessary refuge for roaches; however, foundresses have large mandibles and can crush roaches with them. In foundress chambers, roaches were observed repeatedly moving just below the foundress' mandibles, a precarious position (**Figure 1.5**). Presumably, roaches were attracted to this area by the tiny bit of garden cared for by the foundresses. This suggests a counterintuitive possibility: incipient colonies may be more hostile environments (i.e., represent more "resistant" hosts) than established colonies, even though incipient colonies lack workers (Castella et al. 2009). Incipient colonies that possess enough fungal garden to attract but not protect roaches may make roaches especially vulnerable to foundress attacks. Also, if fungal gardens facilitate roach chemical mimicry (Nehring et al. 2016), the lack of it could make roaches conspicuous as intruders to aggressive foundresses in their tiny nests.

Interestingly, while foundresses were observed expressing antagonism toward roaches in the survivorship experiment, including biting and removing attached roaches (self-grooming), queens in the multi-chamber nests appeared to tolerate roaches, including in cases where multiple roaches were observed simultaneously riding a queen. Without workers, foundress aggression toward fungivorous intruders such as springtails and mites (Castaño-Meneses et al. 2017), and in this case roaches, could be especially important for protecting incipient gardens. Once workers take over defense tasks and garden care, and the foundress transitions into a specialized egg-laying machine (queen), perhaps her aggression toward intruders subsides.

In the survivorship experiment, it is unclear what killed roaches in incipient colonies (**Figure 1.2**), but the vast majority did not appear to die by foundress attack as their cadavers were initially observed intact and apparently undamaged. Dessication may have been a dominant mortality factor, possibly the byproduct of a lack of fungal garden. Regardless of the means of death, the rapid mortality of *A. fungicola* in *A. texana* foundress chambers suggests that *A. fungicola* are unlikely to persist through the earliest stages of colony development in the field. This result agrees with observations of Sanchez-Pena (2005, p. 12), who reported that the maximum survivorship of four *Attaphila* (sp. unreported) individuals placed in artificial brood chambers with *A. mexicana* foundresses was 15 days, and also agrees with observations of Alex Mintzer who has been unsuccessful at rearing *A. fungicola* with *A. mexicana* foundresses collected in Organ Pipe Cactus National Monument (personal communication).

The following is a major caveat regarding the results of the survivorship experiment: The artificial fungal garden chamber treatment represents a single trial (n = 1, **Table 1.2**). All roaches in this treatment were placed in the same container with the same garden, and the higher survivorship of *A. fungicola* in this garden may reflect access to more and higher quality resources (e.g., greater volume of fungus, benefits of conspecific or *A. texana* workers, etc.) in a laboratory garden compared to expected survivorship under natural conditions in the field.

Even if roaches "infecting" incipient colonies under natural conditions tend to survive as long as their host foundresses do, incipient colonies should still represent low quality hosts compared to established colonies. Among ants, *Atta* species have some of the highest mortality estimates for foundresses, and their incipient fungal gardens fail readily (Jacoby 1944; Fowler 1987; Cole 2009; Marti et al. 2015). Unless *A. fungicola* mediate challenges faced by foundresses not explored in this study (e.g., allo-grooming of *A. texana* foundresses to help remove pathogens), or preferentially attach to female alates during mating flights with above-average nest-founding success, roaches that remain with foundresses during early stages of colony development should suffer mortality rates at least as high as their hosts.

If incipient colonies are extremely low-quality hosts as their fragility and high mortality suggest, vertical transmission to incipient colonies should be disadvantageous for roaches. Yet roaches hitchhike on and display a strong preference for female alates, a phoretic behavior that suggests roaches are vertically transmitted. A potential resolution to this “paradox” is that roaches hitchhike on female alates to reach established colonies, not incipient colonies. In other words, roaches may use female alates as vectors for horizontal transmission to high quality established colonies rather than for vertical transmission to low quality incipient colonies. I investigate this possibility in Chapter 2.

CHAPTER 2

Emigrating together but not establishing together: A cockroach rides ants and leaves

Abstract[2]:

Symbionts of ant colonies can hitchhike on winged ant reproductives (alates) during colony nuptial flights. *Attaphila fungicola* Wheeler, a miniature cockroach that lives in the nests of Texas leaf-cutter ants (*Atta texana* Buckley), hitchhikes on female alates (winged queens). Hitchhiking roaches are presumably vertically transmitted from leaf-cutter parent colonies to daughter colonies, remaining with female alates as they transition into foundresses (workerless queens); however, foundresses have limited resources and high mortality rates. Rather than remaining with foundresses likely to die (vertical transmission), roaches might abandon them during dispersal to infect higher-quality later stages of colony development (female alate–vectored transmission). In field experiments, I find evidence for female alate–vectored transmission and discover that roaches use a second hitchhiking step (riding foraged plant material) to infect established colonies. This work reveals a novel relationship between host dispersal and symbiont transmission and shows that colony development can be an important selection pressure on transmission.

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[2] This chapter and supplementary videos are published as Phillips, Z. I. (2021). Emigrating together but not establishing together: A cockroach rides ants and leaves. *The American Naturalist*, 197, 138–145.

INTRODUCTION

Parents that are large, long-lived, and sessile often produce tiny dispersing offspring that tend to die quickly. Trees release seeds, corals broadcast larvae, and ant colonies issue female alates (winged queens). After emigrating from their parents, these propagules attempt to establish in appropriate habitats, develop to maturity, and reproduce; however, most individuals die as immatures (Moles and Westoby 2004; Graham et al. 2008; Marti et al. 2015).

Before emigrating from their parents, propagules can inherit symbionts (collectively, parasites, mutualists, and commensals). For example, seeds can inherit microbes from parent plants (Gitaitis and Walcott 2007), and female alates can inherit microarthropods (e.g., mites) from parent colonies (Campbell et al. 2013). By infecting propagules, these symbionts can (1) acquire their hosts' offspring as new hosts (vertical transmission) or (2) use propagules as ephemeral dispersal agents to reach other hosts (herein,“ propagule-mediated transmission”). In the first case a propagule functions as a new home for a symbiont, while in the second case a propagule functions as a relatively brief mode of transportation. Note that “vertical transmission” here refers only to the first case, in which an inherited symbiont persists as an infection at least through the entirety of a propagule’ s dispersal, from emigration to establishment.

Differences between host life-history stages (e.g., juveniles vs. adults) in attributes that influence host quality, such as mortality rates, resources, and immunity (Combes 2001; Bolnick et al. 2003; Rynkiewicz et al. 2015; Ashby and Bruns 2018; Iritani et al. 2019) may affect whether symbionts evolve vertical transmission or propagule-mediated transmission. In particular, for symbionts restricted to a single host species, these differences may limit vertical transmission and facilitate a distinct mode of intraspecific propagule-mediated transmission. To wit, if early stages of host development following propagule establishment (e.g., juveniles) are lower-quality hosts than later stages (e.g., adults), symbionts may benefit from bypassing juveniles in favor of infecting adults. As a result, symbionts might evolve to exploit propagules as dispersal agents for reaching adult hosts rather than for vertical transmission to juvenile hosts. In other words, symbionts might use host propagules specifically as vectors for transmitting between conspecific adult hosts. Symbiont encounters with adults during propagule dispersal could be facilitated by host intraspecific competition and cannibalism (e.g., adults consume the infected dispersing propagules of conspecific individuals; Van Allen et al. 2017), symbiont manipulation of host propagules (Choisy et al. 2003; Weinersmith 2019), abandonment of propagules during dispersal (Camargo et al. 2015), and shared habitat preferences between propagules and adults (Fowler et al. 1986; Edmunds 2000; Levine and Murrell 2003; Slater et al. 2010).

In this study, I explore whether *Attaphila fungicola* Wheeler, a symbiotic cockroach of leaf-cutter ant colonies and their mutualist fungal gardens, uses propagule-mediated transmission to move between host colonies. A benefit of studying this system is that symbiont behavior can be observed in the field during key stages of host propagule dispersal. In Texas, *Attaphila fungicola* has a single available host species, the Texas leaf-cutter ant (*Atta texana* Buckley). The roach feeds on the ants' mutualist fungal garden (Nehring et al. 2016; Z. I. Phillips, personal observations) but likely has negligible fitness effects on established colonies (for life-history and ecology details, see Wheeler 1900, 1910; Moser 1964; Waller and Moser 1990; Nehring et al. 2016; Phillips et al. 2017; Djernæs et al. 2020). The roach has traditionally been assumed to use winged leaf-cutter female alates exclusively for vertical transmission (**Fig. 2.1**) from parent colonies to daughter foundresses (wingless, workerless queens beginning new colonies; Moser

1967*a*; Waller and Moser 1990; Phillips et al. 2017; Djernæs et al. 2020). The roaches hitchhike on female alates during the ants' nuptial flights and display a preference for female alates over male alates (male winged ants) in choice tests (Phillips et al. 2017); however, the delicate, high-mortality foundresses that female alates become after nuptial flights are likely much lower-quality hosts than long-lived, resource-rich established colonies (Waller and Moser 1990; Little et al. 2003; Baer et al. 2009; Vieira-Neto and Vasconcelos 2010; Mueller et al. 2011; Marti et al. 2015).

After nuptial flights, female alates regularly land in habitats with established conspecific colonies (Fowler et al. 1984, 1986; Fowler 1987; U. G. Mueller, personal communication; Z. I. Phillips, personal observations). As a consequence, roaches may have evolved to abandon female alates and foundresses after nuptial flights in order to infect larger established colonies. I call this taxon-specific mode of propagule-mediated transmission "female alate-vectored transmission" (**Fig. 2.1**), and I use the term to refer to any event where female alates facilitate the dispersal of ant colony symbionts (i.e., myrmecophiles) between established colonies of the same host species. To determine whether female alate-vectored transmission occurs, I test the following predictions: (1) roaches abandon female alates and foundresses, (2) roaches can use colony foraging lines to "infect" established colonies, and (3) individual roaches can both hitchhike on female alates and use foraging lines to infect established colonies.

METHODS

Attaphila fungicola females and *Atta texana* female alates were collected during nuptial flight preparations of mature leaf-cutter colonies using methods described in Phillips et al. (2017). The roaches and ants were collected in 2018 (May 5, May 16, May

21) and 2019 (April 25, May 3, May 5). On all dates, mature colonies were sampled at Brackenridge Field Laboratory, Austin, Texas (n = 5 in 2018, n = 4 in 2019), except on May 16, 2018, when mature colonies were also sampled at Hornsby Bend Environmental Research Center in Austin, Texas (n = 6). Foundresses were collected in parking lots adjacent to and nearby Brackenridge Field Laboratory on May 4, 2019 (n = 18), and May 5, 2019 (n = 34), as well as at Hornsby Bend on May 9, 2019 (n = 46). No *A. fungicola* were found attached to foundresses (n = 98). Data have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.d51c5b00h; Phillips 2020). *Attaphila fungicola* specimens collected (accession number: UTIC255785) and not lost or destroyed during these and other experiments are accessioned at the Insect Collection of the University of Texas at Austin (https://biodiversity.utexas.edu/collections).

To test whether *A. fungicola* remain with leaf-cutter foundresses as they found new colonies (i.e., as they search the ground for a spot to begin excavation and as they excavate their initial brood chamber), recently captured *A. fungicola* were coupled with recently captured foundresses, and each foundress was released with a single attached *A. fungicola* (n = 24 in total, from May 5 and 9, 2019) onto a portion of dirt path at the Brackenridge Field Laboratory where foundress excavations have been observed. Foundress-*A. fungicola* pairs were released in intervals to allow observations to last up until each foundress completed excavation or its attached *A. fungicola* absconded, and experiments lasted from approximately 9 a.m. to 5 p.m. each day. To facilitate foundress excavation behavior, shallow depressions were dug into the soil of the path before the experiments.

To test *A. fungicola*'s ability to directly infect established colonies, individuals were placed on foraging trails of mature nonnatal *A. texana* colonies (n = 2) during nocturnal foraging. *Attaphila fungicola* collected from each nuptial flight were used in field tests (n = 59 in total; n = 8 from May 5, 2018; n = 25 from May 16, 2018; and n = 26 from May 21, 2018). Each *A. fungicola* was placed individually in an open vial on a foraging trail less than 5 m from a leaf-cutter nest/foraging trail entrance, and all foraging trails were actively moving leaf fragments during field tests.

To test whether *A. fungicola* can move from vectoring female alates to foraging trails and subsequently use foraging trails to infect established colonies, female alates each with a single attached *A. fungicola* (n = 22 in total; n = 6 from April 25, 2019; n = 16 from May 3, 2019) were placed on active foraging lines of the only mature leafcutter colony still engaged in diurnal foraging at Brackenridge Field Laboratory, located in a sidewalk margin between the field lab's perimeter fence and an adjacent public road.

RESULTS

Eighteen foundresses began excavations on the path; four foundresses began excavations off the path, including at the bases of nest mounds formed by invasive fire ants (*Solenopsis invicta*); and two did not begin excavations during the observation period. All *A. fungicola* abandoned foundresses either before excavations began (n = 7) or after excavations began but before they were complete (n = 17). Four pairs of cofoundresses formed (i.e., a newly released foundress joined a previously released foundress in excavating the same hole), and all roaches abandoned foundresses after they became cofoundresses (n = 4). After abandoning foundresses, 16 *A. fungicola* moved and remained under nearby litter for at least 5 min, five entered cracks in the soil and constructed partial barriers of tiny compactions of soil, and three were lost to observation. *Attaphila fungicola*'s excavation of soil has not been observed before. Observations of dispersal distance were limited. Foundress excavations and locations where individual *A.*

fungicola were last observed were flagged and inspected the day after foundress release. Only four foundresses were recovered from foundress chambers. No *A. fungicola* were recovered from these chambers or from the locations where they were last observed. At this time, one foundress was observed beside an open excavation hole being attacked by leaf-cutter workers.

Fifty-four of the *A. fungicola* introduced independently to foraging lines attached to leaves carried by passing leaf-cutter workers. Forty-one of these individuals appeared to antennate at least one passing worker without a leaf or any quarry and did not attach to these workers. Five *A. fungicola* attached directly to workers without leaves, and these ants appeared to become agitated and move erratically off of foraging trails. Thirty four of the *A. fungicola* that attached to leaves were directly observed being carried into leaf-cutter nest/foraging trail entrances. When a leaf-cutter forager dropped a leaf being ridden by an *A. fungicola* individual, the roach soon attached to a passing leaf carried by a different worker (15 observations). *Attaphila fungicola* deposition of ootheca (egg cases) on leaves or workers was not observed, and all *A. fungicola* remained within the apparent bounds of the foraging trail when unattached to passing workers and/or their foraged leaves.

All roaches abandoned female alates introduced to foraging trails, either as the alates were attacked by workers (n = 14) or after alates moved off of foraging trails (n = 8). After abandoning alates, 10 roaches subsequently hitchhiked on foraged material, and 12 moved under nearby litter. None were observed constructing soil barriers, which may have been due to the dry, hardened substrate of the foraging trails and surrounding area. Foragers carried leaves and flowers, and roaches were observed hitchhiking on both.

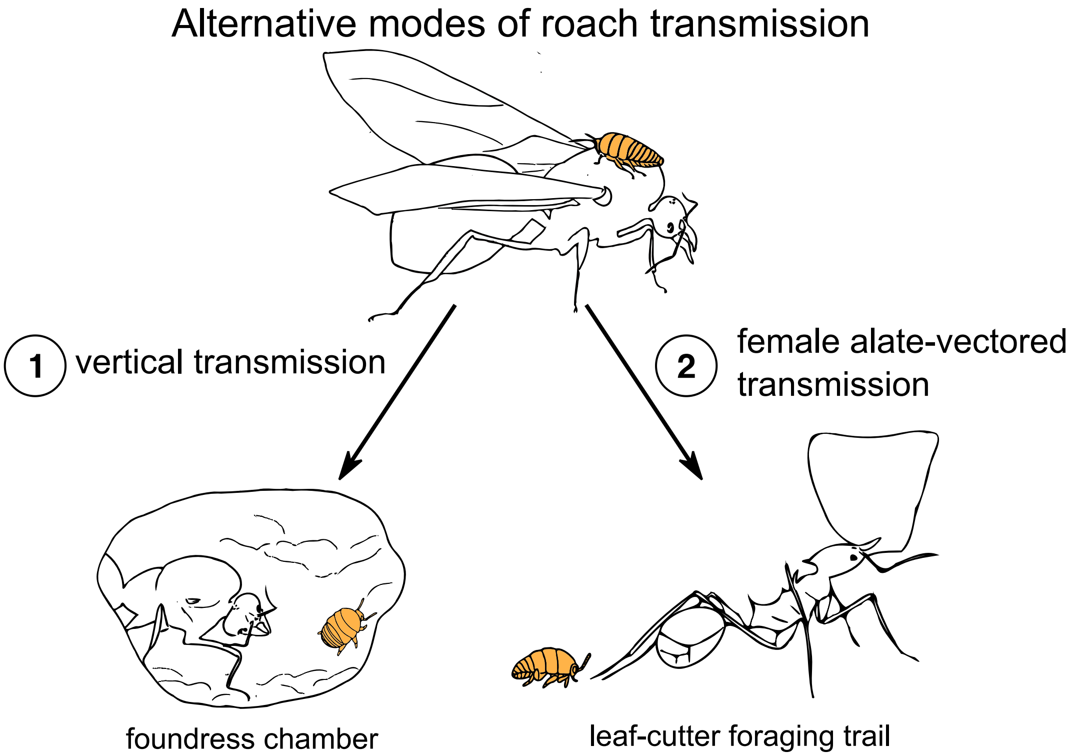

Figure 2.1: Alternate modes of female alate-dependent roach transmission.

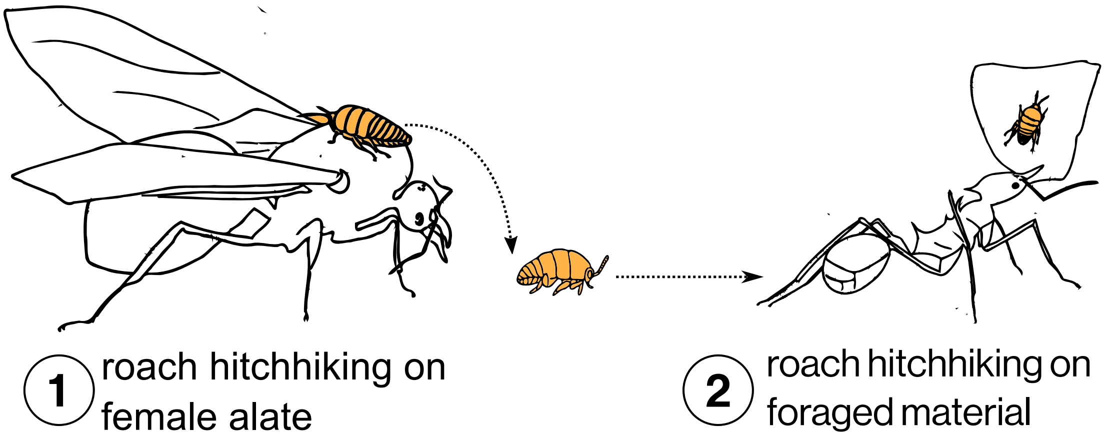

Figure 2.2: The "Texas Two-step."

DISCUSSION

Host development can have a profound effect on transmission dynamics (Briggs and Godfray 1995). If this general relationship extends to the scale of the superorganism (i.e., coevolved society of individuals), we can expect host colony development to influence the between-colony transmission of symbionts (Wilson 1985; Cremer and Sixt 2009; Tschinkel 2011; Kennedy et al. 2017; Quevillon et al. 2018). For example, a leaf-cutter colony develops from an ant into an expansive superorganism (Hölldobler and Wilson 2009, 2011; Marti et al. 2015). The prodigious differences between the vulnerable foundresses that initiate colonies and the resilient superorganisms that they become should affect the transmission of some colony symbionts. In particular, symbionts may evolve transmission strategies that bypass foundresses in order to infect higher-quality established colonies.

In this study, I find that the symbiotic cockroach *Attaphila fungicola* exhibits two behaviors that facilitate female alate-vectored transmission (**Fig. 2.1**) but not vertical transmission (**Fig. 2.1**): first, the roaches abandon leaf-cutter foundresses; and second, the roaches hitchhike on plant material carried by foragers. Thus, despite having apparently easy access to foundresses *A. fungicola* has evolved a mode of transmission that bypasses the foundress stage of colony development.

The opposing movement of female alates during nuptial flights (away from the nest) and plant material carried by foragers (toward the nest) suggests that roaches can benefit from hitchhiking twice on a journey between colonies (**Fig. 2.2**, the “ Texas two-step”). They can use female alates as initial vectors for emigrating from upstream host colonies and use returning foragers as final vectors for infecting downstream host colonies. It would be counterproductive for roaches to hitchhike on foraged material in order to emigrate from an upstream host colony – this would promote the roach' s return to the same host colony.

As a general form of dispersal, the Texas two-step resembles diplochory, a seed dispersal strategy defined as "seed dispersal by a sequence of two or more steps or phases, each involving a different dispersal agent" (VanderWall and Longland 2004, p. 155). The roach' s two dispersal agents are leaf-cutter female alates and foragers. For other phoretic arthropods (Baumann 2018), including those not associated with ants, similar dispersal strategies characterized by sequential phoresy may be common but overlooked. Like the roaches, other phoretic arthropods might use primary dispersal agents to reach certain habitats (e.g., habitats with leaf-cutter ants) and secondary dispersal agents to reach particular microhabitats maintained by ecosystem engineers (e.g., leaf-cutter nests; Hastings et al. 2007).

The roach' s preference for female alates over male alates (Phillips et al. 2017) may be due to the superior vectoring ability of females, not their capacity to produce new colonies and facilitate roach vertical transmission. Male alates are much smaller than female alates (Moser 1967*a*; Moser et al. 2004; Helms IV 2018) and are likely less capable of hauling roaches on nuptial flights. Also, males lack purpose after they mate midair with female alates (Boomsma et al. 2005; Heinze 2016); the postcoital aimlessness of males could set them and their passengers adrift, carrying roaches to unfavorable destinations.

Although no roaches have been confirmed collected in foundress chambers in Texas (Phillips et al. 2017) and none were observed attached to foundresses in surveys conducted for this study (see "Field Survey and Collection"), Moser (1967*a*, p. 304) reports roaches in " new burrows made by queens," suggesting vertical transmission between parent and daughter colonies (**Fig. 2.2**). The roaches may also be able to move directly between colonies (i.e., alate-independent transmission), but *A. fungicola* has never been observed leaving a nest unattached to a female alate, and the mature nests of *Atta texana* are relatively spread out and do not share foraging territories (e.g., approximately 0.2 nests/ha at Brackenridge Field Laboratory). In any case, it remains unclear how roaches initially encounter foraging trails.

In Central and South America, *Attaphila* roaches live in the nests of both *Atta* and *Acromyrmex* leaf-cutter ants (Bolívar 1901; Brossut 1976; Sanchez-Pena 2005; Rodriguez et al. 2013; Nehring et al. 2016) and are promising candidates for studying the evolution of between-colony transmission (Mueller et al. 2017; Quevillon et al. 2018). Across the roaches' geographic range, variation in environmental factors and host phenotypes has likely selected for different transmission strategies. Greater host colony densities and higher humidity in certain regions could promote the evolution of direct, alate-independent modes of transmission. Differences in the phenology and organization of nuptial flights (Moser 1967*a*; Stürup et al. 2011) and in the nuances of collective foraging (Waller 1989; Moll et al. 2013) could also influence roach dispersal behaviors. For instance, smaller workers that hitchhike on larger foragers in some leafcutter species (Linksvayer et al. 2002) might prevent roaches from catching rides on foraging trails.

Among ants, it is not uncommon for female alates and foundresses to be dragged into the colonies of conspecifics after nuptial flights and killed or adopted (Glancey and Lofgren 1988; Hölldobler and Wilson 1990; Evans 1996; Souza et al. 2005). As a consequence, a broad range of organisms that ride female alates may regularly skip the foundress stage of colony development and end up in larger established colonies. This could be a particularly important dispersal route for soil microarthropods (Robin et al. 2019; Lubbers et al. 2020), key members of soil ecosystems that form a variety of specialized and opportunistic associations with ants (Sleptzova and Reznikova 2006;

Vandegehuchte et al. 2015; Castaño Meneses et al. 2017). For example, mite myrmecophiles (i.e., ant associates) are extremely abundant and diverse (Berghoff et al. 2009; Uppstrom and Klompen 2011; Lachaud et al. 2016; Parmentier 2020) and include species that disproportionately attach to female alates (Campbell et al. 2013).

In general, a better understanding of how symbionts exploit host propagules for dispersal and transmission can improve predictions of symbiont range expansion (Mestre et al. 2020). For example, parasite adaptations for propagule-mediated transmission, if traded off with adaptations for vertical transmission (Zilio et al. 2018), could facilitate instances of enemy release (Liu and Stiling 2006; Roy et al. 2011), whereby invasive organisms escape parasites from their native range. A parasite that exclusively undergoes propagule-mediated transmission between adult hosts and is not vertically transmitted could not simultaneously colonize a habitat with host propagules; in the new habitat, susceptible adult hosts would not be available. As a consequence, the parasite’ s range expansion could lag behind that of its host, and host populations at the range front could in turn be released from the parasite. This example shows, in principle, one way in which adaptations for emigrating with – but not establishing with – host propagules may influence regional and global distributions of symbionts.

Since roaches can use female alates for horizontal transmission, it seems likely that their dominant route of transmission is horizontal, not vertical. I investigate this possibility in Chapter 3.

CHAPTER 3

The early life of a leaf-cutter ant colony constrains vertical transmission and favors horizontal transmission

Abstract[3]:

Colonial organisms host a large diversity of symbionts (collectively, parasites, mutualists and commensals) that use vertical transmission (from parent colony to offspring colony) and/or horizontal transmission to disperse between host colonies. The early life of some colonies, characterized by dispersal and establishment of solitary individuals, may constrain vertical transmission between parent and incipient daughter colonies and favor horizontal transmission between large established colonies. We explore this possibility with the miniature cockroach *Attaphila fungicola*, a symbiont of leaf-cutter ants and the mutualist fungal gardens they cultivate. Based on roach co-dispersal behaviors during leaf-cutter nuptial flights (i.e., hitchhiking on winged queens), *A. fungicola* has traditionally been assumed to use vertical transmission between parent leaf-cutter colonies and their daughter incipient colonies; however, weak compatibility between roaches and incipient fungal gardens may constrain roach co-establishment with incipient colonies and thus vertical transmission. Reciprocally, opportunities for horizontal transmission between larger established colonies may weaken selection against roach-induced harm (virulence) of incipient fungal gardens. We use a lab experiment, behavioral observations, field surveys and a transmission model to estimate the effect roaches have on the survivorship of incipient fungal gardens and the frequency of roach vertical transmission. Contrary to traditional assumptions, our results indicate that roaches harm incipient fungal gardens and predominantly use horizontal transmission between established leaf-cutter colonies. Ultimately, “costs of generalism” associated

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[3] This chapter and supplementary videos are published as Phillips Z.I., L. Reding and C.E. Farrior (2021) The early life of a leaf-cutter ant colony constrains vertical transmission and favors horizontal transmission. *Ecology and Evolution*, (11)17, 11718–11729.

with infecting disparate stages of a colony’s lifecycle (e.g., incipient and established colonies) may constrain the vertical transmission of roaches and a broad range of symbionts.

INTRODUCTION

> Slowly something began to trickle into my brain: organisms are not just adults – they are lifecycles.
>
> – John Tyler Bonner, *Life Cycles: Reflections of an Evolutionary Biologist*

Colonial organisms represent important habitat patches of biodiversity, hosting diverse populations of parasites, mutualists and commensals (herein, collectively referred to as “symbionts”). Although mature colonies can become enormous, colony lifecycles often commence with tiny solitary forms (Yang 2007; Hölldobler and Wilson 2009; Marti et al. 2015; Martin et al. 2018), and these humble beginnings can play a crucial role in the relationship between colonies and their symbionts. In particular, the early lives of colonies can influence patterns of between-colony transmission, constraining vertical transmission and favoring horizontal transmission. For example, theory predicts that beneficial symbionts (zooxanthellae) of stony corals should be vertically transmitted from parent to offspring corals (Bull et al. 1991; Herre et al. 1999; Bennett and Moran 2015; Hartmann et al. 2017, 2019); however, the same symbionts that benefit larger, settled corals can harm their tiny dispersing larvae (Hartmann et al. 2017, 2019). For some corals, these larval-specific costs of infection favor coral acquisition of symbionts from the environment (horizontal acquisition) rather than from parents (vertical transmission), deviating from theoretical predictions that do not account for coral lifecycle heterogeneity (Hartmann et al. 2017, 2019).

For symbionts of eusocial insects, the solitary early life of a colony may present similar challenges to vertical transmission (herein, transmission from parent colony to incipient daughter colony), and accounting for colony lifecycle heterogeneity could dramatically alter predictions of transmission dynamics. Many colonies of ants, bees, wasps, and termites begin with just one or a few individuals and expand into colonies of thousands or millions of members, with resources growing in kind from meager to abundant (Hölldobler & Wilson, 2009; Tschinkel, 2011; Wheeler, 1910; Wilson, 1985). Leaf-cutter ants in the genus *Atta* exemplify this transformation. They begin with one or a few gynes (female reproductives) each carrying a pellet of mutualist fungus and develop into complex insect societies maintaining abundant fungal gardens (Forti et al., 2017; Marti et al., 2015). Notably, the gyne is the only ant that passes through the entire colony lifecycle, including the solitary incipient stage. She changes in form and function from a winged gyne dispersing from her parent colony on a nuptial flight (female alate) into a wingless, workerless gyne raising an incipient colony (foundress), and finally, after the eclosure of her first brood of workers, she becomes the queen of the colony's ergonomic and mature stages (i.e., growth and reproductive stages, respectively)(Fernández-Marín & Wcislo, 2005; Marti et al., 2015; Wilson, 1985).

At the scale of the colony, changes in form and function result from the codependent processes of fungal garden cultivation (Mueller et al., 2017) and sociogenesis, “the process by which colony members undergo changes in caste, behavior, and physical location incident to colonial development,” (Wilson, 1985, pp. 1489) creating a dynamic within-nest environment for symbionts. During the lifecycle of a colony, these “guests” can experience changes in their host colony's size, resources, defenses, nest architecture, interaction networks, and other qualities that affect symbiont fitness (Cremer & Sixt, 2009; Hughes et al., 2008; Parmentier, 2020; Rynkiewicz et al., 2015; Tschinkel, 1993; Woodard et al., 2013). As such, colony stage-dependent variation, including traits specific to the early life of a colony (Moreira et al., 2019), should be consequential for symbiont ecology and evolution.

We explore how the early life of a leaf-cutter colony affects the between-colony transmission of *Attaphila*, symbiotic cockroaches that exploit the ants and their mutualist fungal gardens (Bohn et al., 2021; Bolivar, 1901; Brossut, 1976; Djernæs et al., 2020; Nehring et al., 2016; Rodríguez et al., 2013; Waller & Moser, 1990; Wheeler, 1900). In Texas and Louisiana, *Attaphila fungicola* Wheeler is ostensibly common in the established colonies (i.e., ergonomic and mature) of its only available host, the Texas leaf-cutter ant (*Atta texana* Buckley) (Moser, 1964, 1967a, 2006; Nehring et al., 2016; Phillips et al., 2017; Waller & Moser, 1990). Moser reports that "the roach inhabits the fungus gardens of most nests," but does not provide a specific estimate of prevalence (1964, pp. 1048). At our field sites in Austin, TX, mature leaf-cutter colonies with chronic roach infections (>5 years) survive and reproduce apparently unimpaired, so we use the neutral term "symbiont" instead of "parasite" to describe them (Phillips, 2021); however, the effect of roaches on incipient colony survival is unknown.

During the mass upheaval of a colony nuptial flight, roaches hitchhike on a small proportion of their host colony's dispersing female alates (<7%), and typically each "infected" female alate bears a single phoretic female roach (Moser, 1967*a*; Phillips, 2021; Phillips et al., 2017; Waller & Moser, 1990). Hitchhiking (i.e., co-dispersal) on female alates has traditionally been interpreted as a behavior that initiates vertical transmission, likely because it suggests roaches remain with female alates as they become foundresses, and that roaches then co-establish with foundresses and their incipient colonies (Djernæs et al., 2020; Moser, 1967*a*, 1967b); however, there is no evidence that roaches persist as infections through the incipient stage of colony development.

Furthermore, recent findings indicate that hitchhiking roaches can abandon female alates after nuptial flights and subsequently ride leaves carried by foragers into the nests of established colonies (Phillips, 2021). Accordingly, hitchhiking on female alates may facilitate a complex mode of horizontal transmission between established colonies ("female alate-vectored transmission," Phillips, 2021) rather than vertical transmission to incipient colonies. In other words, roach co-dispersal with female alates can be uncoupled from roach co-establishment with foundresses and incipient colonies, and it remains unclear how frequently co-establishment and thus vertical transmission occurs.

The low host quality of incipient colonies (extremely high mortality, low tolerance for disturbance, meager incipient gardens) may limit roach co-establishment and constrain vertical transmission, favoring routes of horizontal transmission that bypass incipient colonies (direct or female alate-vectored transmission between established colonies) (Moser, 1964; Phillips, 2021). Reciprocally, if roaches rarely or never use routes of vertical transmission that pass through incipient colonies, selection on roaches to avoid overexploiting and damaging incipient gardens should be weak (weak incipient garden compatibility). Alternatively, if roaches rely heavily on vertical transmission for dispersing between nests, they should be under strong selection to successfully co-establish with incipient colonies, and to minimize harm and possibly provide benefits to incipient gardens (strong incipient garden compatibility) (Combes, 2001; Genkai-Kato & Yamamura, 1999; Iritani et al., 2019; Lipsitch et al., 1996). To test whether roaches exhibit strong or weak incipient garden compatibility, and whether roaches primarily use vertical or horizontal transmission, we use a laboratory experiment to estimate the effect individual roaches have on the survivorship of low-volume fungal gardens in artificial foundress chambers, and we use field surveys and a between-colony transmission model to estimate the contribution of vertical transmission to roach prevalence among mature leaf-cutter colonies.

METHODS

Attaphila fungicola female roaches and *A. texana* female alates were collected during nuptial flight preparations of mature leaf-cutter colonies in May 2018 at Brackenridge Field Laboratory, Austin, TX (30.2840°N, 97.7780°W) (May 5, 21) and Hornsby Bend, Austin, TX (30.2327°N, 97.6374°W)(May 16). As thousands of alates and many thousands of nestmates gathered on nest mounds early in the morning, researchers ventured onto the mounds to collect alates and hitchhiking roaches. Eight out of 11 sampled mature *A. texana* colonies were infected with roaches (73% mature colony "infection" prevalence: 3/5 mature colonies with roaches at BFL, 5/6 mature colonies with roaches at Hornsby Bend). In total, 420 roaches were collected from 7,791 female alates (an average of 5.5% of female alates from colonies with roaches had a single roach attached). Roach prevalence per infected colony ranged from 2.2% to 6.8% of female alates bearing a single hitchhiking roach. Data have been deposited at the Dryad Data Repository (Phillips et al., 2021; https://doi.org/10.5061/dryad.8sf7m 0cnt). Specimens of *A. fungicola* collected and not lost or destroyed during these and other experiments are accessioned (Accession number: UTIC255785) at the Insect Collection of University of Texas at Austin (https://biodi versity.utexas.edu/collections).

Incipient garden survivorship experiment

We collected paired female alate ants and roaches for use in the experiment, where alates collected from the field already had attached *A. fungicola* roaches. Using naturally paired ants and roaches ensured that both species came from the same natal colony, and thus controlled for potential intercolony differences (e.g., chemical profiles). We removed the wings of the female alates and placed the de-winged alate (herein, “foundress”) and her attached roach in a 5 cm diameter container (“foundress chamber”) with 20 mg of fungal garden (“incipient garden”) from a laboratory colony. Notably, 20 mg is larger than the inoculum of fungus that foundresses initially regurgitate when founding a new colony under natural conditions (Marti et al., 2015). Long-term survival in the laboratory of foundresses provided only with their inoculum is extremely rare, complicating experimentation and highlighting the extreme fragility of incipient colonies.

We compared the survivorship of incipient gardens and foundresses in two treatments: (a) foundress with roach treatment (i.e., foundress “infected”) and (b) foundress without roach treatment (i.e., foundress “uninfected”). We conducted the experiment after two nuptial flights from Brackenridge Field Laboratory (Flight 1: n = 53, Flight 2: n = 43, total n = 96 roach-foundress pairings). Experiments were conducted under laboratory conditions described in Phillips et al. (2017), with all replicates kept at room temperature (22–24°C). Chambers were checked for 1 min every 24 hr in low-light conditions to determine mortality of fungal gardens, foundresses, and roaches. The fungal garden was marked as effectively dead if it was dismantled and scattered in decaying clumps in upper and/or lower corners of the chamber and if the foundress did not tend any portion of the garden for at least 30 s (“uncaring” foundresses), or if the foundress was dead (without a caretaker, the fungal garden is effectively dead). Alternatively, the fungal garden was marked as living if the foundress tended a contiguous portion of the garden for at least 30s (i.e., the foundress’ head and mandibles maintained a position facing and over the garden, typically manipulating and antennating it. Additionally, 30 min following initial set-up, each foundress chamber was observed for 3 min to determine if the inhabiting roach disturbed the incipient garden. Garden disturbance was scored if roach contact caused any observable movement, physical dislocation, or fragmentation of the garden, and subsequently, we categorized gardens as either "disturbed" (one or more observations of roach disturbance) or "undisturbed" (no observations of roach disturbance).

To test the effect of *A. fungicola* on incipient garden mortality, we used a mixed-model Cox proportional hazards model with right-censored daily mortality as the response variable and treatment (presence or absence of *A. fungicola*) as the explanatory variable. Nest location was nested within flight date as a random effect to account for variation in survivorship between flight dates and nests. Survival analyses were run using version 2.2 of the coxme package and the survival R package (Therneau, 2015). Additionally, we used a chi-square test to determine whether there was a difference in the proportion of dead foundresses between the roach-present and roach-absent treatments. All analyses used R version 4.0.1 (R Core Team, 2020).

Between colony transmission model

To explore the maximum contribution of vertical transmission to roach infection prevalence among mature colonies, we develop a simple model that assumes exclusive vertical transmission. We use this model to estimate mature colony infection prevalence (V) from foundress infection prevalence (J) and the effect of roach presence on the likelihood of a foundress reaching the mature colony stage (δ). See **Figure 3.1** for a diagram of the model, and **Table 3.1** for parameter symbols and definitions.

Parameter δ is the net effect of roach presence on foundress and incipient colony survivorship. A value of $\delta < 1$ indicates that the roach is harmful to foundresses and their incipient colonies, $\delta = 1$ that the roach is neutral, and $\delta > 1$ that the roach is beneficial. To estimate δ from our incipient garden survivorship experiment, we use the inverse of a hazard ratio calculated from our survivorship analysis. This is a dimensionless measure of the effect roaches have on incipient garden survivorship.

Our field estimate of foundress infection prevalence, J, is not directly based on foundress infection prevalence (i.e., co-establishing roaches) because roaches have not been observed in incipient colonies in our study region (Phillips et al., 2017) and we are not aware of estimates of foundress infection prevalence in any other region and for any other *Attaphila* species. This makes our best direct estimate of J zero and would indicate that vertical transmission does not occur. To account for the possibility that we have not observed these rare events, including the possible deposition of roach ootheca (egg cases) on alates or in foundress chambers, we estimate the maximum potential value of J from the maximum proportion of female alates with hitchhiking roaches collected from a single mature *A. texana* colony. This is likely a highly conservative estimate of J given that roaches co-establishing with foundresses seem much rarer than roaches co-dispersing with female alates (see Section 4). The maximum prevalence of roaches on a single nest mound in Austin, TX that has been recorded is 0.07 (On 15 May 2016, 50/719 female alates collected from a single nest mound surface preparing for nuptial flights at Brackenridge Field Laboratory). As we describe in “Model Results” below, using this conservative estimate of $J = 0.07$ helps estimate a conservative maximum possible contribution of roach vertical transmission to mature colony infection prevalence (V).

Model description

Our model is composed of four classes of ants: (a) foundress with roach (i.e., “infected” foundress) (F_i); (b) foundress without roach (i.e., “uninfected” foundress) (F_u); (c) mature colony with roach (i.e.,“infected” mature colony) (M_i); (d) mature colony without roach (i.e.,“uninfected” mature colony) (M_u). (Note, “infection/infected” here refers to the presence of a roach and/or its progeny in a host colony, not to microbial infections).

Mature colonies with roaches (M_i) are generated in our model by the development of foundresses with roaches (F_i) that survive to colony maturity with their roach infection intact. This is determined by the rate that foundresses with roaches reach the mature colony stage (s_i) and the persistence of inherited roaches through colony development (z). If we assume mature colonies with roaches die at rate q, the change of mature colonies with roaches over time is as follows:

$$\frac{dM_i}{dt} = F_i s_i z - M_i q \quad . \tag{Eq. 1}$$

Mature colonies without roaches (M_u) are generated by foundresses without roaches that survive to colony maturity (F_u), determined by the rate that foundresses without roaches reach the mature colony stage (s_u), and by the rate that foundresses with roaches (F_i) lose their inherited roaches and reach the mature colony stage ($s_i(1 - z)$).

$$\frac{dM_u}{dt} = F_u s_u + F_i s_i (1 - z) - M_u q. \tag{Eq. 2}$$

We assume that foundresses with roaches that lose them during colony development (e.g., the roaches die) are as likely to reach colony maturity as foundresses that maintain roaches through colony development (s_i). In other words, we assume that the likelihood of foundresses reaching the mature colony stage is independent of the duration of roach infections. This assumption is consistent with our experimental results, which indicate *A. fungicola* has a rapid effect on low-volume fungal garden survivorship (**Figure 3**). We also assume that mature colonies with roaches die at the same rate as mature colonies without roaches (q). This assumption is based on observations of similar nest surface frequencies of *A. fungicola* (i.e., similar proportions of female alates with hitchhiking roaches during nuptial flight preparations of a given infected nest: roughly 2%–7%) over a span of 5 years with no apparent reduction in colony health or size of nuptial flights. This assumption is also consistent with the general prediction that symbionts of large, long-lived colonies are likely to evolve relatively low virulence (Hughes et al., 2008).

Given these assumptions, the mature colony infection prevalence (V), foundress infection prevalence (J), and the effect of *A. fungicola* on the likelihood of a foundress reaching colony maturity (δ) are defined as the following, respectively:

$$V \equiv \frac{M_i}{M_i + M_u} \qquad \text{(Eq. 3)}$$

$$J \equiv \frac{F_i}{F_i + F_u} \qquad \text{(Eq. 4)}$$

$$\delta \equiv \frac{s_i}{s_u}\,. \qquad \text{(Eq. 5)}$$

By solving Equations 1 and 2 at equilibrium, and using the above relationships (Equations 3 – 5), we find V defined as a function of δ, J and z.

$$V = \frac{\delta z}{{}^{1}\!/_{J} - 1 + \delta} \qquad \text{(Eq. 6)}$$

We use Eq. 6 to answer the following question: What is the maximum proportion of mature colonies that could be infected through the vertical transmission of roaches to foundresses (V_{max})? In other words, what is the maximum proportion of mature colonies that could acquire roaches that co-disperse with female alates, then co-establish with foundresses, and subsequently persist as colony infections until colony maturity? First, we estimate V_{max} based on our laboratory estimate of the roach effect on incipient fungal garden survivorship ($\delta = 0.3$, the inverse of hazard ratio 3.36, see Section 3). Second, we estimate V_{max} under the conservative assumption that roaches have no effect on incipient fungal garden survivorship (neutral, $\delta = 1$). By "conservative assumption," we specifically mean an assumption that selects parameter values deviating from more realistic values (i.e., values based on experiments, surveys, or natural history observations) in a way that maximizes model estimates of V.

For all estimates, we make the conservative assumption that roaches are never lost or cleared after occupying an incipient colony's foundress chamber ($z_{max} = 1$). In nature, $z < 1$ is certainly more accurate. Foundresses, for instance, have been observed attacking and killing roaches (Phillips et al., 2017). As discussed above, our estimate of J from female alate infection prevalence ($J = 0.07$) is likely a significant overestimate of foundress infection prevalence and therefore likely inflates our model estimates of V.

Note: We do not include the difference equations for infected foundresses (F_i) and uninfected foundresses (F_u) because they do not alter the result of Eq. 6 derived from the difference equations for infected and uninfected mature colonies (see Equations 1 and

2, respectively). The parameters *g* (*g* = number of foundresses produced/mature colony) and *p* (*p* = proportion of infected foundresses produced/mature colony) included in the model diagram also do not alter the result of Equation 6; we assume the production of female alates does not directly effect changes in the number of mature colonies. The model excludes male *A. fungicola* because they are generally absent during nuptial flights (Phillips et al., 2017; Waller & Moser, 1990). It has been proposed that *A. fungicola* are parthenogenetic in Louisiana (Waller & Moser, 1990) where no male *A. fungicola* have been collected from A. texana colonies.

RESULTS

Attaphila fungicola has a negative impact on the survivorship of incipient gardens when both “uncaring” and dead foundresses are included in the category of nonsurviving gardens (Figure 3; hazard ratio = 3.36, z = 7.01, p < 0.001). If only “uncaring” foundresses are included in the analysis, *A. fungicola* still has a negative impact on the survivorship of incipient gardens (hazard ratio = 4.44, z = 6.68, p < 0.001). The proportion of dead foundresses does not differ between treatments (chi-squared test, X_2 = 0.659, df = 1, p = 0.417). Roaches were observed disturbing incipient gardens in 62.5% of foundress chambers (n = 96, 95% confidence interval: 52.0%–72.2%).

By incorporating our conservative values of z and J ($z = 1$, $J = 0.07$) and our laboratory estimate of δ ($\delta = 0.3$) into Equation 6, we calculate that $V = 0.02$ (2% mature colony infection prevalence). Under these same conditions, but assuming roaches have no effect on the likelihood of a foundress reaching the mature colony stage ($\delta = 1$), we calculate that $V = 0.07$ (7% mature colony infection prevalence). Our field survey estimate of mature colony infection prevalence $V = 0.73$ (73% mature colony infection prevalence). A simple comparison of our model estimate of V based on our laboratory estimate of δ (V = 0.02) with our field survey estimate of V (0.73) indicates that strict vertical transmission could at most produce roughly 3% (0.02/0.73) of the proportion of infected mature colonies surveyed in the field. A similar comparison of our model estimate of V when we assume the roach has no effect on incipient garden survival (V = 0.07) with our field survey estimate of V (0.73) indicates that strict vertical transmission could at most produce about 10% (0.07/0.73) of the proportion of infected mature colonies surveyed in the field. Thus, both conservative model estimates of V indicate that vertical transmission is responsible for at most a small proportion (3% or 10%) of roach prevalence among surveyed mature colonies.

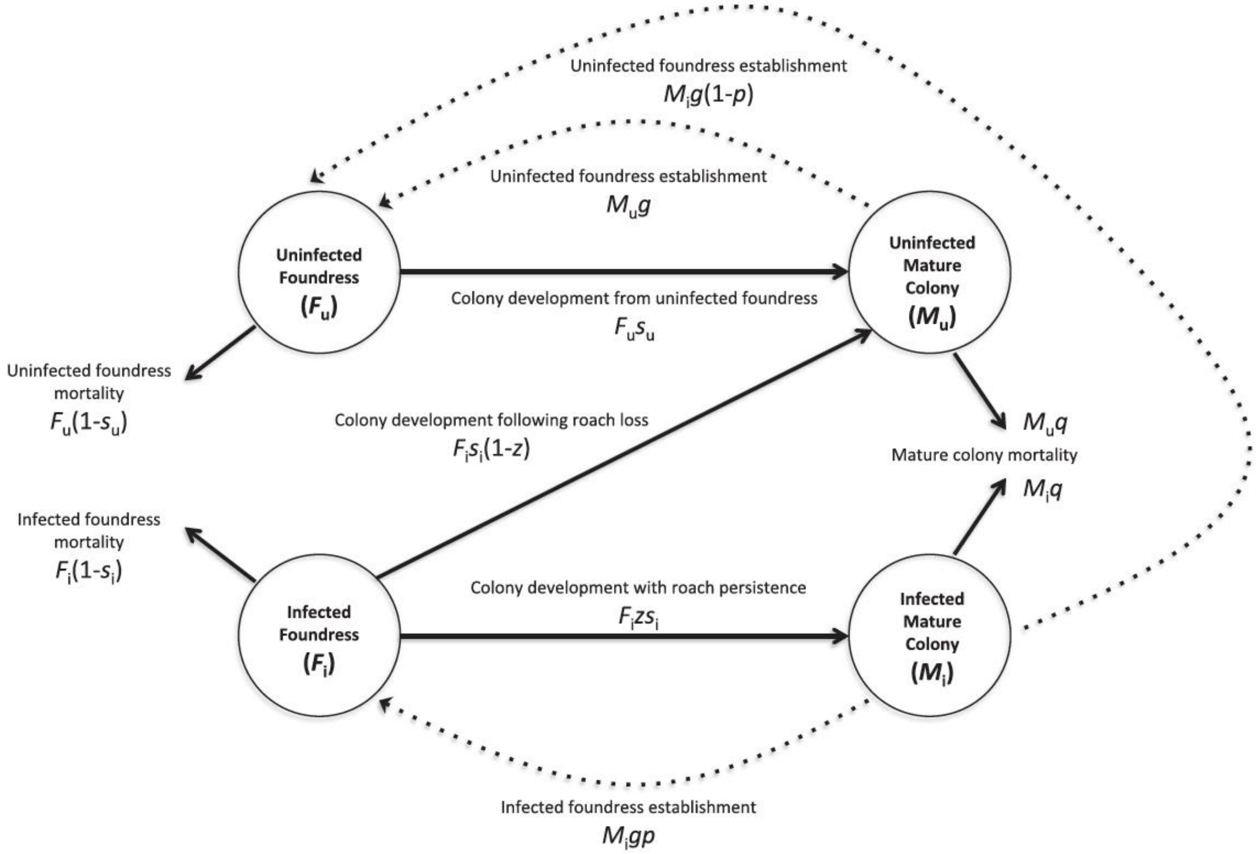

Figure 3.1: Transmission model.

F_u	Number of foundresses (incipient colonies) without roaches (individual)
F_i	Number of foundresses (incipient colonies) with roaches (individual)
M_u	Number of mature colonies without roaches (colony)
M_i	Number of mature colonies with roaches (colony)
s_u	Yearly proportion of foundresses without roaches (F_u) reaching mature colony stage (colony/ind/year)
s_i	Yearly proportion of foundresses with roaches (F_i) reaching mature colony stage (colony/ind/year)
$\delta = s_i/s_u$	Roach effect on the likelihood of foundress reaching the mature colony stage (unitless)
z	The persistence of inherited roaches across colony development, from foundress host to mature colony host (unitless)
q	Mortality rate of mature colonies (1/year)
V	Mature colony infection prevalence (i.e., proportion of mature colonies with roaches); $M_i/(M_i + M_u)$ (unitless)
J	Foundress infection prevalence (i.e., proportion of foundress chambers occupied by roaches); $F_i/(F_i + F_u)$ (unitless)

Table 3.1: Model symbols and definitions

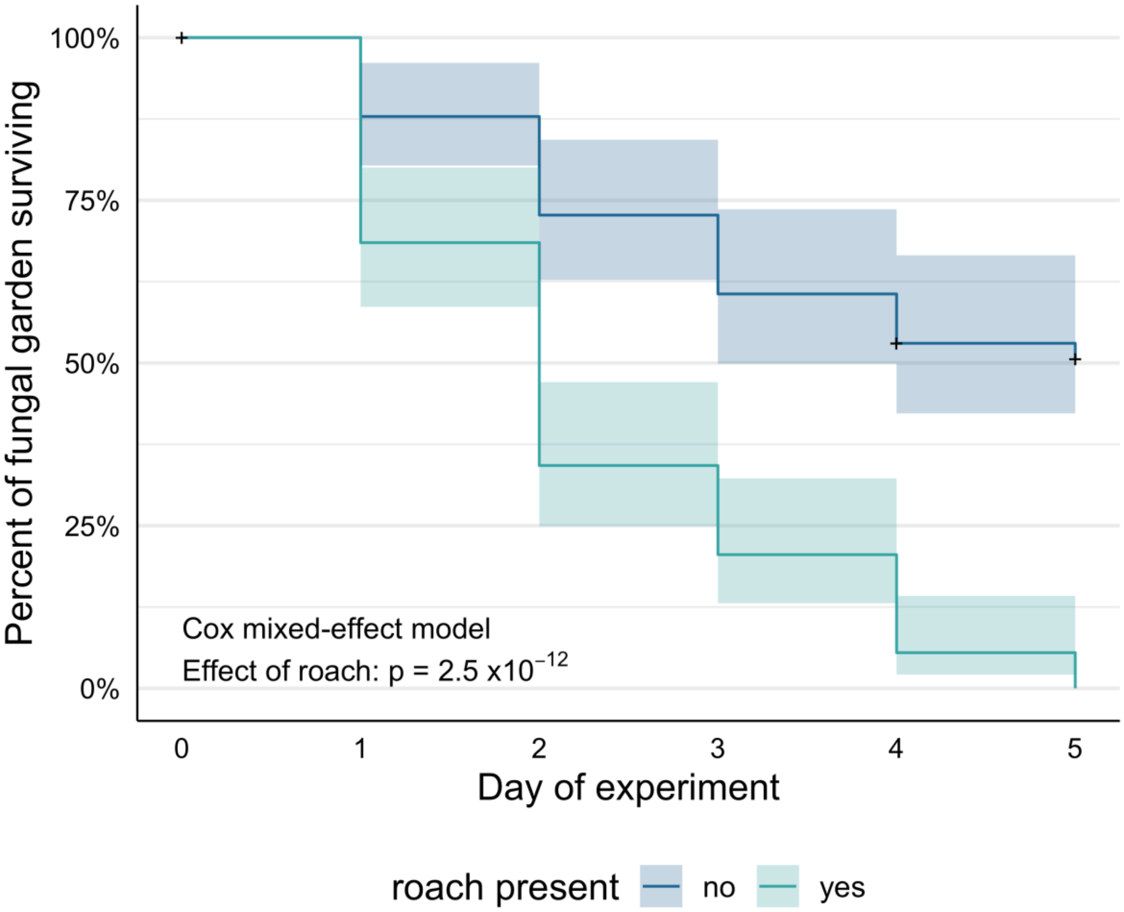

Figure 3.2: Survivorship of incipient fungal gardens in the presence or absence of *Attaphila* cockroaches.

DISCUSSION

Symbionts inherited by host propagules (e.g., plant seeds, coral larvae, ant gynes) must co-disperse and co-establish with propagules for vertical transmission to be successful (Bibian et al., 2016). As a consequence, accounting for challenges that symbionts face during both host dispersal and establishment can help identify constraints on vertical transmission.

The propagules of leaf-cutter ant colonies are gynes and the mutualist fungus they carry and care for, and the early life of a colony is marked by their dispersal (winged female alates carrying fungal pellets) and establishment (workerless foundresses raising incipient fungal gardens) (Helms, 2018; Marti et al., 2015; Moser, 1967a). During leaf-cutter nuptial flights, the symbiotic cockroach *A. fungicola* hitchhikes on female alates (co-dispersal), a behavior that strongly suggests roaches are vertically transmitted to incipient colonies (Moser, 1967a; Waller & Moser, 1990); however, roach co-establishment with foundresses may be limited by weak compatibility with incipient gardens. The collective results of our experiment, behavioral observations, field surveys, and model indicate that roaches are weakly compatible with incipient gardens, that they at most rarely use vertical transmission, and that they primarily use horizontal transmission between established colonies.

Given the extreme fragility of incipient fungal gardens, we would expect selection for compatibility with incipient gardens to be strong for any vertically transmitted symbiont and for such symbionts to avoid harming or to even benefit incipient gardens during co-establishment (Fries & Camazine, 2001; Genkai-Kato & Yamamura, 1999; Herre et al., 1999; Lipsitch et al., 1996). In contrast, our results suggest roaches have evolved fixed responses to robust gardens rather than plastic behaviors that can be attuned to delicate gardens. In artificial foundress chambers, we observed roaches feeding on and rubbing against gardens (the latter may help the roaches acquire a colony's chemical profile)(Nehring et al., 2016), behaviors that are likely harmless to established gardens but could be catastrophic to incipient gardens and responsible for their accelerated failure (**Fig. 3.2**). Also, roaches appeared to stress foundresses, consistent with observations from a previous study (Phillips et al., 2017). By antagonizing a foundress, a roach could indirectly cause significant damage to the garden (Moreira et al., 2019) (note, these interactions were observed but not scored as disturbances in our “incipient garden survivorship experiment” because they do not involve direct contact between roach and garden). Lastly, roaches might act as vectors of “hyperphoretic spores” and microbial garden diseases that kill incipient gardens (Di Prisco et al., 2011; Hughes et al., 2004; Moreira et al., 2019; Moser & Blomquist, 2011).

Mixed-mode transmission between colonies

Attaphila fungicola vertical transmission may occur rarely, with populations of roaches using both vertical and horizontal transmission (i.e., mixed-mode transmission)(Antonovics et al., 2017; Ebert, 2013). The only field observation that ostensibly describes roaches co-establishing with incipient colonies notes *A. fungicola* in “new burrows made by [A. texana] queens” (Moser, 1967a, pp. 304). Other field observations suggest co-establishment and vertical transmission are rare. Roaches have not been collected from *A. texana* foundress chambers in central Texas (Phillips et al., 2017), nor in incipient nests of its sister species *Atta mexicana* in Organ Pipe Cactus National Monument, AZ, and attempts to have these roaches co-establish with foundresses have been unsuccessful (Phillips et al., 2017; pers. communication Alex Mintzer). Also, in northern Mexico, individuals of an unidentified species of *Attaphila* were observed running around independently of nearby *A. Mexicana* foundresses searching for nest sites (Sánchez-Peña, 2005) suggesting that these roaches had abandoned foundresses before vertical transmission could be completed (i.e., roach co-dispersal uncoupled from co-establishment).

If *Attaphila* roaches exhibit mixed-mode transmission, perhaps encounters with foundress predators (e.g., armadillos, grackles, myrmecologists) influence a roach's decision to either take a chance remaining with a foundress likely to die (vertical transmission) or abandon the foundress and risk seeking an established colony (female alate-vectored horizontal transmission). In north Texas, a roach jumped off of a foundress seeking a nest site and disappeared into the grass, an escape apparently prompted by a researcher's collection of the foundress (U. G. Mueller, personal communication). Also, it is possible that roaches deposit ootheca (egg cases) on female alates or with foundresses before abandoning them; however, in field experiments where roaches were released while attached to female alates and foundresses, this was not observed (Phillips, 2021). In another study, roaches deposited ootheca within a few days of being collected with female alates during nuptial flight preparations, suggesting ootheca deposition would not have occurred during the nuptial flight itself (Waller & Moser, 1990). Even in the unlikely scenario that every hitchhiking roach attaches an ootheca to its co-dispersing female alate, or deposits an ootheca during co-establishment with a foundress, and assuming that ootheca and potentially accompanying adult roaches are harmless to incipient gardens, our model predicts vertical transmission would still occur infrequently (under these conservative conditions, our model predicts that the maximum proportion of mature colonies infected through vertical transmission is 10%).

Overall, the disparity between high mature colony prevalence and low female alate and foundress prevalence of roaches suggests vertical transmission is rare—unless roaches are somehow beneficial to incipient colonies and colonies with roaches disproportionately reach maturity. Our experiment indicates no such mutualism occurs (**Fig. 3.2**). Infrequent vertical transmission could still play an important role in roach population dynamics and evolution, and vertical transmission might occur at higher frequencies in areas where the density of established leaf-cutter colonies is low and there are fewer opportunities for horizontal transmission (e.g., range frontiers) (Mueller et al., 2011). Also, *Attaphila* individuals, "strains" and species could vary in their compatibility with incipient colonies, creating within-species and between-species variation in the frequency of vertical transmission. A comparative analysis of transmission strategies among *Attaphila* might reveal conditions that facilitate vertical transmission, but we know little about the life histories of most species or how many species exist (Bohn et al., 2021; Bolivar, 1901; Brossut, 1976; Djernæs et al., 2020; Nehring et al., 2016; Rodriguez et al., 2013; Sánchez-Peña, 2005; Wheeler, 1900).

Potential roach strategies for mitigating virulence during co-establishment

Virulence (i.e., symbiont-induced harm to a host) can be adaptive or nonadaptive for symbionts (Bull, 1994; Leggett et al., 2013). A common model of adaptive virulence frames it as a property emerging from the trade-off between transmission period and transmission rate: Increasing within-host reproduction is costly because it increases virulence and reduces symbiont transmission period (i.e., kills the host faster), but beneficial because it increases symbiont transmission rate (i.e., rate of infection of new hosts) (Bull, 1994; Day, 2003). In contrast, the roach-induced harm observed in our experiment probably represents nonadaptive virulence (**Fig. 3.2**). A Texas leaf-cutter foundress raises her incipient colony in a closed-off (“claustral”) underground chamber (Marti et al., 2015), the same small space a vertically transmitted roach would presumably occupy during co-establishment. If a roach contributes to the death of an incipient garden, it likely seals the fate of itself and its progeny in a shared grave with garden and foundress.

Nonadaptive virulence can be described as “virulence of no selective value per se…a coincidental byproduct of [symbiont] evolution in a different host species” (Bull, 1994, pp. 1424–1425). As this suggests, a major cause of nonadaptive virulence is infecting the “wrong” host, a host that a symbiont has not co-evolved with and may not be compatible with (i.e., a host outside of the symbiont's host range) (Bull, 1994; Combes, 2001; Leggett et al., 2013). Although the “wrong” host often refers to an incompatible host species or strain, here we use it to refer to a potentially incompatible colony lifecycle stage, the incipient colony, which for *Attaphila* represents a radically different host environment than an established colony. Under this premise, roach behaviors that harm incipient colonies could arise as a byproduct of roach co-evolution with established colonies. For example, if roaches have evolved an adaptive attraction to fungal gardens in the garden-rich environment of established colonies, the same attraction may be nonadaptively virulent when expressed in the garden-poor environment of incipient colonies. As discussed above, roaches might be able to mitigate this harm by adjusting their behavior during co-establishment to avoid incipient gardens (i.e., behavioral plasticity, Leggett et al., 2013), or by exclusively using “behavior-less” ootheca to co-establish with incipient colonies (i.e., ontogenetic niche shift, ten Brink & de Roos, 2017; Werner & Gilliam, 1984).

Additionally, roaches may be able to mitigate harm by targeting leaf-cutter co-foundresses instead of solitary foundresses. Leaf-cutter foundresses can join together to start a new colony, and these co-foundress collectives exhibit higher survivorship and produce larger incipient gardens than solitary foundresses (Cahan & Julian, 1999; Mintzer, 1987). As a consequence, co-foundresses and their incipient gardens could exhibit a greater tolerance for roaches (Ayres & Schneider, 2012; Cremer et al., 2018; Pull et al., 2013), increasing the likelihood of both roach and incipient colony survival; however, roaches have been observed abandoning co-foundresses during the excavation of new colonies (Phillips, 2021; Phillips et al., 2021), suggesting that if roaches do infect incipient colonies, targeting co-foundresses may not be a preferred strategy.

In general, abundant resources during co-establishment should reduce the risk of symbiont overexploitation and catastrophic damage. Consider the early life of an ant plant-homopteran mutualism, one in which a sap-sucking scale insect (the homopteran) co-establishes with an ant foundress on a myrmecophytic tree (Gaume et al., 1998). Although scale insects can be vectors of disease (Brown, 2016), and infestations can damage host plants (Golan et al., 2015), the sap-sucking of one or a few scale insects during co-establishment is unlikely to mortally wound a tree and doom the tripartite symbiosis. Now imagine if leaf-cutter foundresses initiated colonies with tree-sized fungal gardens instead of seed-sized fungal gardens. Presumably a roach in this scenario would be innocuous during co-establishment regardless of its behavior, and vertical transmission would not be constrained by incipient gardens.

Costs of generalism may constrain vertical transmission

Vertical transmission from parent to daughter incipient colonies requires both roach encounters with and compatibility with incipient colonies (Combes, 2001). Hitchhiking on female alates (co-dispersal) facilitates encounters with incipient colonies because roaches simply have to remain with female alates as they transition into foundresses. Indeed, these easy encounters seem to be the basis for assuming hitchhiking is a first step in vertical transmission, and that co-dispersal is tightly linked to co-establishment; however, vertical transmission also requires compatibility with both incipient colonies and established colonies, while horizontal transmission requires compatibility with only established colonies. In the first case (vertical transmission), a roach must be a "generalist" of host colony lifecycle stages, while in the latter case (horizontal transmission), a roach can be a "specialist" of just established colonies. As a consequence, costs of generalism may ultimately constrain vertical transmission, not horizontal transmission, and attenuate the link between roach co-dispersal and co-establishment.

Some authors have divided costs of generalism that constrain symbiont compatibility with distinct hosts (i.e., restrict host range) into two categories: ecological costs and evolutionary costs (Benmayor et al., 2009; Leggett et al., 2013). Symbionts are susceptible to ecological costs when their potential hosts vary in quality, a scenario "analogous to that assumed in optimal foraging theory, where patches vary in quality" (Benmayor et al., 2009, pp. 764). In this context, ecological costs for symbionts are opportunity costs that result from infecting lower quality hosts instead of higher quality hosts (Bull, 2006; Heineman et al., 2008). Incipient leaf-cutter colonies are intrinsically lower quality hosts than established colonies, possessing much higher mortality rates, fewer resources, and a lower tolerance for disturbance than established colonies. As such, we would expect vertical transmission from parent to daughter incipient colonies, but not horizontal transmission between established colonies, to impose strong ecological costs of generalism on roaches. Evolutionary costs of generalism associated with roach vertical transmission are less clear. Evolutionary costs arise if a symbiont's ability to infect one kind of host (the “novel host”) is associated with reduced performance in another kind of host (the “original host”)(Benmayor et al., 2009; Leggett et al., 2013). These costs could result from antagonistic pleiotropy between roach traits enhancing performance in incipient colonies (e.g., avoidance of fungal garden) and traits enhancing performance in established colonies (e.g., attraction to fungal garden).

Although many insect societies found colonies with just one or a few individuals (“independent founders”), some found colonies with a large number of individuals (“dependent founders”) (Cronin et al., 2013; LeBrun et al., 2013; Vargo & Porter, 1989). Army ants reproduce through a process called “colony budding,” in which a new queen accompanied by a large group of nestmates break off from their parent colony to form a new colony (Cronin et al., 2013). Budding allows an army ant colony to effectively skip the incipient stage and begin its life as an established colony (Cronin et al., 2013; Denny et al., 2004; Kronauer et al., 2010; Rettenmeyer et al., 2011). As a consequence, the vertical transmission of army ant symbionts entails transmission from one established colony (parent) to another (daughter) and should be less constrained by costs of generalism than the vertical transmission of leaf-cutter symbionts such as *Attaphila*. Consistent with this possibility, albeit without invoking costs of generalism, Berghoff et al. (2009) and Łukasik et al. (2017) argue that army ant colonies should be more susceptible than independent founders (e.g., leaf-cutters) to inheriting colony symbionts such as phoretic mites (Berghoff et al., 2009) and socially transmitted microbes (Łukasik et al., 2017).

If leaf-cutter colonies were to reproduce through colony budding as army ants do, how would this affect *Attaphila* transmission? The vertical transmission of *Attaphila* might be less constrained by costs of generalism, and daughter colonies would likely inherit roaches more frequently.

Concluding remarks

Colonies are lifecycles, and many begin with just one or a few individuals. From the perspective of a colony symbiont, the solitary early life of a colony represents a radically different host environment than that of a large established colony. Compared to established colonies, incipient colonies possess few resources, succumb easily to disturbance, and suffer high rates of mortality. All else being equal, incipient colonies are lower quality hosts than established colonies, and infecting the former instead of the latter can be costly. Across a broad range of host-symbiont systems, these costs may constrain routes of vertical transmission that pass through incipient colonies and favor routes of horizontal transmission that bypass them.

CHAPTER 4

Notes on an invasive ant-mimicking spider, *Falconina gracilis*

Abstract:
Falconina gracilis Keyserling is an invasive ant mimic from South America that feeds on ants and associates with nests of ants and their foraging columns. Although the spider is a widespread invasive species, its biology remains obscure, possibly due to its nocturnal and myrmecophilic habits. In Austin, Texas USA, I regularly observed *F. gracilis* at night near leaf-cutter ants to address the following questions: (1) Is *F. gracilis* prevalent among colonies of the Texas leaf-cutter ant *Atta texana*? (2) How does *F. gracilis* feed on ants? (3) Where does *F. gracilis* deposit its eggsacs? (4) Does *F. gracilis* usurp the nests of foundresses?

INTRODUCTION

Although *F. gracilis* has a close relationship with nocturnally active leaf-cutter ants in South America (Fowler 1981, 1984), in Texas it has been reported in association with invasive fire ants (*Solenopsis invicta* Buren) (Valle et al. 2013; Dean 2016) and not with the Texas leaf-cutter ant *Atta texana*. Specifically, in addition to feeding on leafcutter ants, the spider occupies nest entrances and follows foraging columns of *Acromyrmex* leaf-cutters in Paraguay (Fowler 1981). A similar relationship with nocturnally active Texas leaf-cutters could arise through "ecological fitting" (Agosta and Klemens 2008) whereby traits evolved by *F. gracilis* to exploit leaf-cutter ants in South America facilitate exploitation of ants in Texas. Consistent with this hypothesis, I have observed *F. gracilis* occupying the nest entrances and following the foraging columns of Texas leaf-cutter ants at night.

Although Fowler (1981) reports *F. gracilis* predation on *Acromyrmex* leaf-cutter ants, he does not describe any particular mode of feeding. In Austin Texas, I have observed *F. gracilis* along leaf-cutter foraging lines carrying ants in a “kiss of death” position, in which the spider holds the ant with its chelicerae (“jaws”) by the ant’s mouthparts (**Figure 4.1).** Ant heads contain protein-rich tissues targeted by predators (Pekár et al. 2010), but the thick cuticle protecting the head can be difficult to breach, so an ant’s mouth – including its membranous mouthparts – may provide *F. gracilis* easy access to the high-value tissues within.

Falconina gracilis is not an obligate associate of leaf-cutters and can exploit other ants and microhabitats (Valle et al. 2013). As I was unable to find *F. gracilis* eggsacs on or around nest mounds of *A. texana* (March – July 2019), I resorted to flipping over rocks (June 2020). Under rocks along walking paths at Brackenridge Field Lab, I found solitary *F. gracilis* females guarding eggsacs attached to rock undersurfaces. Some females took refuge in retreats resembling the recently excavated nests of *Camponotus* (carpenter ant) foundresses living under nearby rocks. Furthermore, large *Camponotus* body parts, including heads, were scattered around *F. gracilis* retreats or attached to eggsacs (**Figure 4.5**). Combined, these observations suggest that some *F. gracilis* had usurped the nests of foundresses.

METHODS

To estimate *F. gracilis* prevalence among Texas leaf-cutter ant colonies in Austin Texas, I conducted surveys of *A. texana* nest mounds at night. To test if *F. gracilis* feeds on the contents of ant heads and to closely observe *F. gracilis* predation, I conducted feeding trials with leaf-cutter ants and other species. To investigate if *F. gracilis* usurps the nests of *Camponotus* foundresses and deposits eggsacs nearby, I conducted a field experiment at Brackenridge Field Lab.

Surveys of *A. texana* nests mounds for *F. gracilis* were conducted in spring and summer 2019 at night at multiple sites in the Austin area (**Table 4.1**). Nest mounds were inspected for *Falconina gracilis* during periods between 8pm and 5am. Each mound was inspected until a *F. gracilis* spider was observed or for a maximum period of one hour.

Feeding trials with *F. gracilis* adult females (n = 9) and medium-sized leaf-cutter workers were conducted in an Austin apartment in spring 2020 (**Table 4.2**), a version of the "home laboratory" made familiar to many biologists during the Covid pandemic. *F. gracilis* and *A. texana* were collected from Brackenridge Field Lab (30.2843N, 97.7782W) and kept in 5cm diameter round containers (Pioneer Plastics, Inc.) in low light at approximately 25° C room temperature, and remained in isolation with a water source but no food for 5 days before feeding trials. During trials, the duration of "kiss of death" feeding was recorded, and heads were subsequently dissected to determine if head contents were reduced. The start of a bout of "kiss of death" feeding was defined when a spider had maneuvered an ant into "kiss f death" position and the bout ended when the spider released the ant from this position for at least 10 minutes. After spiders had maneuvered ants into "kiss of death" position, they were checked every 5 minutes to determine if they remained that way, and bout duration was rounded to 5-minute marks. The duration of time from *F. gracilis* first bite on ant to when the ant was maneuvered into "kiss of death" position was also recorded (to the minute). An *F. gracilis* individual that did not attack ants within one hour of introduction is not included in the observations.

To investigate if *F. gracilis* usurps the new nests of foundresses, I marked 85 rocks overlaying nests of 91 *Camponotus sansabeanus* foundresses (some rocks overlay multiple nests) along walking paths at Brackenridge Field Lab and surveyed them approximately weekly from mid-May to mid-July, 2021. Additionally, I conducted feeding trials with *F. gracilis* and *C. sansabeanus* foundresses between June 29 – July 18 in an Austin apartment (**Table 4.3**). For these trials, *F. gracilis* females and *C. sansabeanus* foundresses that were not part of regular surveys were collected at BFL, and the same measurements and procedures were used as those in *A. texana* feeding trials.

RESULTS

Falconina gracilis were observed on 14/23 surveyed nests (see **Table 4.1**). In feeding trials, *F. gracilis* typically approached the introduced medium-sized leaf-cutter worker from behind and bit the metasoma (ant gaster; “abdomen”), retreated for a few minutes until the ant was incapacitated, then maneuvered the ant into a “kiss f death” position (**Fig. 4.1**) and remained that way for about an hour to three hours (avg. = 122 minutes; **Table 4.2**). Dissections directly after feeding revealed head capsules to be virtually empty of soft tissues, although in some cases mouthparts were collapsed within the head capsule (6/9 total observations)). After the head, spiders often fed on the mesosoma (“thorax”) and/or metasoma. From 2019 – 2021, I observed the same general predatory sequence when I fed leaf-cutter soldiers and other species of locally collected ants (*Pogonomyrmex barbatus*, *Pseudomyrmex gracilis*) to male and female *F. gracilis*; however, smaller ants (e.g., fire ant minor workers) were typically captured and crushed into boluses without being released from the spider’s grip or maneuvered into a “kiss of death” position.

During surveys of incipient nests of *C. sansabeanus*, five *C. sansabeanus* foundresses were replaced by pale *F. gracilis* in May. The paleness may indicate a recent molting (i.e., teneral spiders). In one case, a foundress corpse lay just outside a nest occupied by a *F. gracilis* spider (**Fig. 4.3**). In June, three *F. gracilis* females were observed under rocks previously occupied by *C. sansabeanus*, but these spiders did not remain to produce eggsacs. In July, the first eggsacs guarded by *F. gracilis* females were observed (n = 4), as well as unattended eggsacs that appeared to be those of *F. gracilis* (n = 3) (**Fig. 4.2**).

In feeding trials, *F. gracilis* consumed *C. sansabeanus* foundresses/queens using the same predatory sequence described for *A. texana* medial workers (**Table 4.3, Figure 4.4**).

Table 4.1: Survey of *F. gracilis* on *A. texana* nest mounds

Austin Site	Coordinates	2019 survey dates	Number of *A. texana* ant nest mounds surveyed	Number of nest mounds with *F. gracilis*
Hornsby Bend	30.2327N, 97.6374W	5/10/11	6	3
Brackenridge Field Lab	30.2843N, 97.7782W	4/17/18	5	4
Roy G. Guerrero River Metro Park	30.1442N, 97.4218W	4/21/22	4	2
Lady Bird Lake Ann Butler Trail	30.2477N, 97.7181W	4/21/22	4	3
Stengl Lost Pines Biological Station	30.0515N, 97.1021W	6/25/26	1	1
Intersection: Pedernales st. and E. Cesar Chavez st.	30.1515N, 974300W	4/11/12	1	1
Intersection: E. 31st st. and Walling dr.	30.1737N, 97.4406W	4/11/12	1	0
Intersection: Cherrywood rd. and Sycamore dr.	30.1736N, 97.4244W	4/11/12	1	0
Total			23	14

Figure 4.1: *F. gracilis* maneuvering *A. texana* into ”kiss of death” position (photo credits, Alex Wild. Arrangement, Z. Phillips)

Table 4.2: *F. gracilis* feeding trials with *A. texana* medial workers

F. gracilis individual	Duration from first bite to “kiss of death” positioning (minutes)	Duration of ”kiss of death” positioning (minutes)
1	26	195
2	7	115
3	12	70
4	23	145
5	6	45
6	7	235
7	41	40
8	5	160
9	19	90
Average	16	122

Table 4.3: *F. gracilis* feeding trials with *C. sansabeanus* foundresses

F. gracilis individual	Duration from first bite to “kiss of death” positioning (minutes)	Duration of “kiss of death” positioning (minutes)
1	8	210
2	7	375
3	12	325
4	6	260
5	17	245
6	8	305
7	10	250
Average	10	281

Figure 4.2: *Falconina gracilis* female guarding her eggsac on underside of rock (left), and six eggsacs removed from a single rock (right)

Figure 4.3: A pale *Falconina gracilis* female exiting a *Camponotus sansabeanus* nest (upper left) as the spider retreat is poked with a stick (blurry foreground). A*C. sansabeanus* cadaver is just outside the spider retreat (bottom center).

Figure 4.4: *F. gracilis* feeding on a *Camponotus sansabeanus* foundress, with eggsac in background.

Figure 4.5: *Falconina gracilis* eggsac covered with insect body parts, including *Camponotus* head (left), and same eggsac with body parts removed (right).

DISCUSSION

In its invasive range in the USA, *Falconina gracilis* spider may be better adapted to exploit leaf-cutting ants than other ants, a potential outcome of co-evolution with leaf-cutters in South America (Fowler 1981, 1984). If leaf-cutters boost *F. gracilis* populations by providing high quality habitat patches, the ants could promote the spider's success in a variety of environments, including urban centers (e.g., downtown Austin) and nature reserves (e.g., Stengl Lost Pines) (**Table 4.1**).

Some predators that feed on ant heads puncture or remove them from behind. The assassin bug *Ptilocnemus lemur* swings behind ants and punctures the soft "neck" region with its straw-like proboscis (Bulbert et al. 2014) and the phorid fly *Dohrniphora longirostrata* decapitates ants by cutting through the same region with its saw-like proboscis (Brown et al. 2015). The distinct ”kiss of death" feeding of *F. gracilis* may provide advantages that feeding through the "neck" region from behind does not, including greater escape mobility (e.g., lower center of gravity, ants not dragged; Franks et al. 2001) a screen of "heavenward" ant appendages to deter nestmate attacks (some ant-mimicking spiders display ant prey to this end**;** Pekár and Křál 2002); and direct access to high-value fluids in the ant's digestive system in addition to its head contents (superficially similar to trophallaxis solicitation performed by parasites of ants (Hölldobler and Wilson 1990). As the above possibilities imply, the behavior may represent a balance of trade-offs between enemy evasion and feeding strategies – a particularly delicate balance for predators of dangerous prey such as ants (Brodie et al. 2002; Bulbert et al. 2014).

During vulnerable and relatively sedentary periods (e.g., molting, maternal care), *F. gracilis* spiders appear to be a facultative usurpers of new *C. sansabeanus* nests. Given the spider's generalist habits, it can likely usurp the new nests of a variety of ant species.

It is unclear if *F. gracilis* targets foundresses and receives any particular benefit from usurping their nests, or foundress predation and nest usurpation are simply an outcome of generalized *F. gracilis* predation, shelter-seeking and maternal care. Regardless, *F. gracilis* may impact ant community dynamics. For example, open-nest foundresses like *C. sansabeanus* should be more susceptible to *F. gracilis* predation than closed-nest ("claustral") foundresses like Texas leaf-cutters. By supporting *F. gracilis* populations, large colonies of Texas leaf-cutter ants might therefore increase predation on heterospecific open-nest foundresses without increasing predation on conspecific closed-nest foundresses. A similar form of "apparent competition" can occur in birds, where different prey nest types ("open cup" vs. "domed ground" nests) result in asymmetric predation (Hoi and Winkler 1994). In a sense, established leaf-cutter colonies could act as "superspreaders" of *F. gracilis* to vulnerable foundresses of other species.

Differences in foundress size and susceptibility to venom could also bias *F. gracilis* predation among species of foundresses. In spring 2021, I fed four *A. texana* foundresses to adult *F. gracilis* females. *A. texana* foundresses are much larger than *C. sansabeanus* foundresses. The spiders repeatedly bit the *A. texana* foundresses, but none appeared to succumb to *F. gracilis* bites and all lived for at least 24 hours following the initial bites. In comparison, *C. sansabeanus* foundresses were incapacitated by *F. gracilis* within minutes (**Table 4.3**)

Falconina gracilis spiders reside in and around both ant-engineered habitat patches and human-altered landscapes, which begs the question: to what degree do ant-engineered habitat patches facilitate *F. gracilis* colonization of human-altered landscapes, including cities? In an extreme case, ant colonies may act as "island" refugia in otherwise inhospitable urban environments. This scenario represents resource tracking (Agosta and Klemens 2008) whereby *F. gracilis* closely tracks a resource – ants and their environmental modifications – into environments shaped by people. In other words, the spider would be *synanthropic* (human-associated) primarily through its association with colonies of synanthropic ants. Alternatively, *F. gracilis* may have traits attuned to other urban features (e.g., buildings, sidewalks, gardens) and/or types of prey, allowing for more human-dependent expansion into cities (Valle et al. 2013). These are not mutually exclusive alternatives, but their relative importance could vary among cities, habitats and ant communities (e.g., Los Angeles, CA without leaf-cutters vs. Austin, TX with leaf-cutters) and differentially affect the evolution of *F. gracilis* populations. For example, colony resource tracking might limit spider exposure to urban selective regimes, favoring the maintenance of traits used to exploit ants (i.e., *myrmecophilic* traits) and constraining the evolution of synanthropic traits.

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