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Dispersal of *Attaphila fungicola*, a symbiotic cockroach of leaf-cutter ants

Z. I. Phillips[1] · M. M. Zhang[2] · U. G. Mueller[1]

Abstract The myrmecophile cockroach *Attaphila fungicola* lives in the nests of leaf-cutter ants (*Atta texana* and *A. cephalotes*) and uses the female winged reproductives (i.e., female alates) of its host as vectors for the first phase of its dispersal. It is unknown whether *A. fungicola* remain with vectoring *A. texana* females after mating flights and throughout *A. texana* nest founding and subsequent colony development, or if the symbiotic cockroaches disperse to established *A. texana* colonies, either on their own or while still attached to vectoring *A. texana* females. We captured *A. fungicola* attached to *A. texana* female alates as they prepared for mating flights and measured their survivorship in artificial brood chambers with de-alate, recently mated *A. texana* queens and their incipient gardens, and in a non-natal established fungal garden tended by *A. texana* workers. After 13 days, 100% of *A. fungicola* had died in brood chambers with queens, while 100% of *A. fungicola* remained alive in the fungal garden chamber. We tested the feasibility of alternative modes of dispersal to established colonies by placing *A. fungicola* attached and unattached to vectoring female alates in the proximity of an established *A. texana* colony directly after a mating flight, and recorded whether *A. fungicola* entered the non-natal nest. A significantly higher proportion of *A. fungicola* attached to vectoring alates entered the nest compared to unattached *A. fungicola*. We also placed *A. fungicola* attached to vectoring alates in a foraging chamber of a laboratory colony to determine if, once in the nest, *A. fungicola* could navigate to the fungal garden chamber; 100% of *A. fungicola* detached from their vectoring alates and entered the fungal garden chamber. We tested alate preference of *A. fungicola* by placing *A. fungicola* separately in 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. Finally, we report the first record of a male *A. fungicola* collected during a mating flight of *A. texana*. These observations accumulated from field and laboratory studies suggest that *A. fungicola* vectored by *A. texana* female alates may not remain with foundresses, but instead disperse between established colonies through one or more alternative mechanisms. This study helps elucidate a tripartite ant-fungus-cockroach interaction, and provides a foundation for future research on *Attaphila* dispersal and reproductive biology.

Keywords *Attaphila* · *Atta texana* · Dispersal · Inquiline · Myrmecophile · Phoretic · Social parasitism · Symbiosis

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Introduction

Nests of leaf-cutter ants (*Atta* and *Acromyrmex*) are inhabited by diverse arthropods, such as mites, spiders, beetles, flies, moths, and other insects (Waller and Moser 1990; Erthal and Tonhasca 2001; Navarrete-Heredia 2001; Sánchez-Peñaet al. 2003; Krantz and Moser 2012), but the nature of the associations between ant host and arthropod "guests" is not well understood. Frequent guests inhabiting leaf-cutter fungus-garden chambers are *Attaphila*

cockroaches (Wheeler 1910; Moser 1967), a genus of six described species (Rodríguez et al. 2013). *Attaphila* are small (up to 3.5 mm in length), wingless, possess well-developed arolia (i.e., tarsal pads) that aid in attaching to the cuticle of the ants, and appear to use modified maxillary palps to follow the chemical trails produced by leaf-cutter workers (Wheeler 1900; Moser 1964; Brossut 1976). The specialized morphology and behavior of *Attaphila*, and the absence of any observations of free-living individuals (i.e., not in association with leaf-cutter ants) suggest that *Attaphila* is an obligate symbiont of leaf-cutter colonies.

Attaphila fungicola Wheeler (Wheeler 1900) inhabits colonies of *Atta texana* in Texas and Louisiana at the northern range limit of the leaf-cutter distribution (Mueller et al. 2011a, b). *A. fungicola* spends almost its entire life inside the host nest, apparently within the fungal garden chambers tended by the ants (Fig. 1) (Wheeler 1900; Moser 2006; Rodríguez et al. 2013). Nearly all observations of *A. fungicola* outside of the nest have occurred during the early phase of the mating flights of *A. texana* when a colony's winged reproductives (i.e., alates) gather on the nest mound in aggregations of several thousand individuals (Moser 1964, 1967; Phillips and Mueller unpublished observations). During predawn preparations and during the actual

mating flight just before dawn, individuals of *A. fungicola* can be readily observed riding on female alates (Fig. 2) aggregating on the nest mound (Moser 1967) and also on female alates when they depart from their natal mound to begin their mating flight (Phillips and Mueller unpublished observation). We are unaware of any observation of *A. fungicola* riding on male alates.

These accumulated observations suggest that *A. fungicola* use female alates of *A. texana* as vectors to disperse from their natal host colony. However, following successful departure by dispersing *A. texana* female alates, it is unclear whether *A. fungicola* remain with their vectoring *A. texana* females as these aspiring queens attempt to found new colonies, or if *A. fungicola* enter previously established leaf-cutter colonies. Previous observations provide limited support for either possibility. In one report, *Attaphila* was observed "running on the ground…like tiny beetles" (Sánchez-Peña 2005) in close proximity to foundresses of *Atta mexicana* that had alighted away from their natal nest, indicating the cockroaches had detached from the foundresses after the mating flight to disperse independently before nest founding was complete. We are also unaware of any observations of *A. fungicola* attached to nest-excavating foundresses, and we have found so far no *A. fungicola* in approximately 150 incipient *A. texana* nests excavated in Texas (Mueller unpublished observation). In contrast, Moser (1967, p. 304) reports that *A. fungicola* can be found in "new burrows made by queens", but the frequency and location of these observations are not reported.

Although the destination of *A. fungicola* directly after *A. texana* mating flights is unresolved, their likely obligate association with leaf-cutter colonies suggests three non-exclusive alternatives for their dispersal: (1) *A. fungicola* accompany foundresses into their sealed (i.e., claustral) brood chambers, where they remain as the incipient colony develops; (2) *A. fungicola* abandon foundresses soon after mating flights and disperse to the fungal gardens of older, established colonies; and (3) *A. fungicola*

Fig. 1 *A. fungicola* in fungal garden of *A. texana*

Fig. 2 *A. fungicola* attached to *A. texana* female alate

enter older, established colonies, while still attached to vectoring female alates. The costs associated with the first strategy include remaining with young *A. texana* colonies subject to high mortality rates, and the costs of the second and third strategy include the challenge of landing in the proximity of, locating and integrating into established *A. texana* colonies, and specifically into a fungal garden chamber. In this study, as a test of the viability of dispersal mechanism (1), we compared the survivorship of *A. fungicola* captured during mating flights between those placed with foundresses, in isolation, and in a fungus garden maintained by workers. To test dispersal mechanisms (2) and (3), we conducted a field and lab experiment representing these possible strategies used by *A. fungicola* to move from an alighting female alate into the nest and fungal garden of a non-natal established colony. In addition, we conducted the first test of *A. fungicola* preference to associate with either female or male alates of *A. texana*.

Methods

Collection of *Atta texana* and *Attaphila fungicola*

Atta texana alates were collected from a mature colony at Brackenridge Field Laboratory (30.2840°N, 97.7780°W) 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 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. For other experiments, collection and preparation methods varied slightly (see below). All *A. fungicola* used in experiments were either mature females or late-juvenile instars of unknown sex, but we did not distinguish between these two.

Attaphila fungicola and *A. texana* queen survivorship

Atta texana foundresses ($n = 52$) searching for nest sites were collected from a parking lot in Cedar Park, TX (30°19′5.86″N 97°39′34.72″W) on 7 May 2015 and transported to the laboratory. We transferred each foundress into an artificial brood chamber constructed from a 5-dram vial filled 75% with moistened dental plaster. In addition, we constructed an artificial fungal garden chamber by transferring approximately 207 cm³ of fungal garden, including the workers tending it, from a lab colony of *A. texana* into a round translucent plastic container (4 cm × 11 cm diameter). We used a digital microscope camera (Dino-Lite AM3111T) to film interactions between *A. fungicola* and *A. texana* workers in the artificial garden (see Online Resource 1).

Within 48 h of alate capture, we randomly assigned *A. fungicola* 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).

We kept all replicates at room temperature (22–24 °C). Humidity in nest containers was always near 100% because of the moistened plaster. We recorded mortality of *A. fungicola* and *A. texana* queens 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), we dissected the fungal garden to count surviving *A. fungicola* in the Fungal Garden treatment, and we recorded the presence or absence of incipient fungal gardens in brood chambers of the Queen treatment.

Attaphila fungicola dispersal into mature *A. texana* colony (in field)

Atta texana female alates ($n = 514$) and attached *A. fungicola* ($n = 25$) were collected from their natal colony's nest mound at Brackenridge Field Laboratory as the alates aggregated in preparation for a mating flight on the morning of 12 May 2016. Within 1 h of capture, we separated *A. fungicola* into two groups: *A. fungicola* retaining attachment to their original vectoring alates (Attached treatment, $n = 10$) and *A. fungicola* removed from vectoring alates

and placed alone (Solitary treatment, $n=8$). Within 3 h of capture, we introduced individuals from each treatment separately onto the edge of the mound of an established non-natal *A. texana* nest that had also produced a mating flight that morning, and thus was still covered with patrolling workers. For individuals of each of the two treatments (Attached or Solitary), we recorded whether or not *A. fungicola* entered or did not enter an opening on the mound of the established nest during a 3-h observational period.

Attaphila fungicola dispersal into fungal garden chamber of mature *A. texana* colony (in lab)

Atta texana female alates and attached *A. fungicola* were collected as described in the above experiment on the morning of 15 May 2016. Within 1 h of capture, we separated *A. fungicola* 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, we separately introduced individuals from each treatment into an internal, non-fungal garden chamber of a mature laboratory colony 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, we 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 alate preference

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).

Statistical analyses

We generated Kaplan–Meier estimates (Kaplan and Meier 1958) of the survival function for *A. fungicola* and *A. texana* queens using the statistical package R. From the Kaplan–Meier estimates, we used Greenwood's formula (Greenwood 1926) to calculate the confidence interval around the median death time. We used a log-rank test 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, we used the

Bonferroni adjusted significance level of $0.05/3=0.0167$. We used a Pearson's Chi-squared test to evaluate if *A. fungicola* display a bias in attaching to female or male alates. To test our alternative hypothesis that *A. fungicola* attached to queens (Attached treatment) enter the nest at a higher proportion than the unattached *A. fungicola* (Solitary treatment), we used Pearson's Chi-squared test.

Results

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 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). All *A. fungicola* collected were either mature females or late instars, except for one mature male *A. fungicola* captured on 7 May 2015. 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).

Attaphila fungicola survivorship

Table 1 summarizes *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 ($p=4.62\mathrm{e}-13$). *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; however, in the Queen treatment, two individual *A. fungicola* were grasped and crushed to death by queens in their respective brood chambers, suggesting that queen aggression may have been a factor of mortality. All *A. texana* queens produced brood, and no *A. fungicola* oothecae were observed in the experimental nest chambers.

Table 1 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

Survivorship of *Attaphila fungicola* after 17 days when living (a) in a worker-tended fungus garden (Fungal Garden treatment), (b) in association with a nest-founding queen and her incipient garden (Queen treatment), or (c) in isolation (Control). Upper and lower confidence intervals are Kaplan–Meier estimates

Atta texana queen survivorship

In the Queen treatment, the log-rank test showed that there was no significant difference in survivorship between *A. texana* queens with *A. fungicola* absent or present in their brood chambers ($p = 0.913$) (Fig. 3). On the final day of the experiment, 3/20 incipient fungal gardens of the surviving solitary queens were absent (i.e., gardens were either lost or never started) and 7/20 incipient fungal gardens of the surviving queens hosting *A. fungicola* were absent. The presence of *A. fungicola* did not influence the mortality of incipient fungal gardens between queens that hosted *A. fungicola* and queens that did not host *A. fungicola* (Pearson's Chi-squared, $p = 0.2733$).

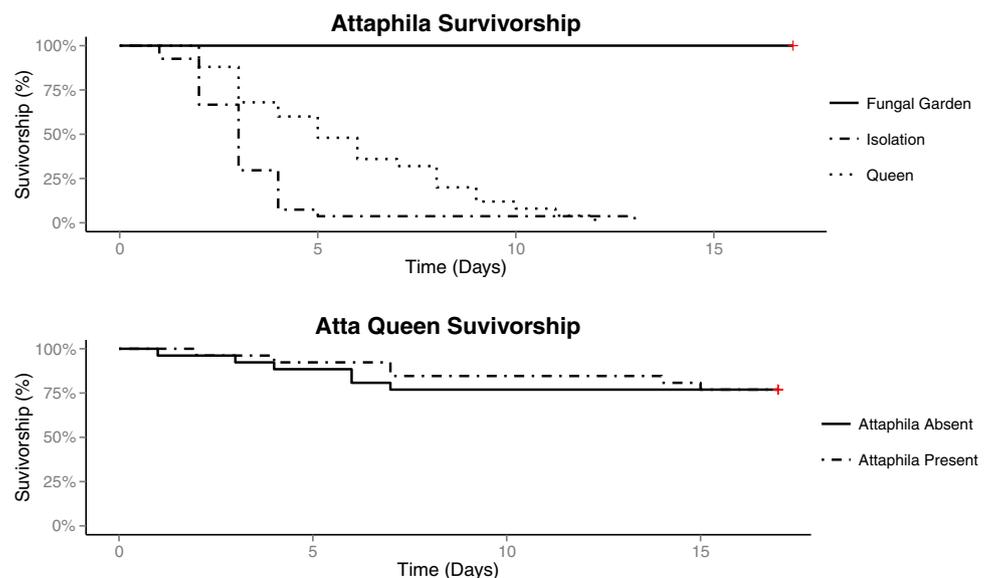### *Attaphila fungicola* dispersal into mature *A. texana* colony (in field)

Of the *A. fungicola* attached to *A. texana* alates (Attached treatment), 8/10 entered the non-natal *A. texana* colony, and 0/8 *A. fungicola* unattached to alates (Solitary treatment) entered the non-natal *A. texana* colony; *A. fungicola*

attached to alates entered nests at a significantly higher proportion than unattached *A. fungicola* ($p = 0.0018$). Upon release at the edge of the nest mound, each solitary *A. fungicola* moved underneath leaf-litter resting on the substrate, and remained until the end of the observational period. The alates with attached *A. fungicola* that entered the nest were attacked by workers en route to the nest entrances, and it is unclear if the alates moved of their own volition or were coerced toward and into the nest entrances by the workers (Online Resource 2).

Attaphila fungicola dispersal into fungal chamber of mature *A. texana* colony (in lab)

100% of *A. fungicola* from both treatments (12/12 Attached, 9/9 Solitary) entered the fungal garden chamber from the adjacent chamber 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

Fig. 3 Survivorship curves for *A. fungicola* in Queen, Fungal Garden, and Isolation treatments, and for *A. texana* queens in Queen Treatment (generated in R and ggplot2)

workers, while the *A. fungicola* appeared to be unharmed (Fig. 3), 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 alate preference

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.

Discussion

This study provides novel observations on the survivorship, alate preference, and dispersal biology of *Attaphila*, a symbiotic cockroach of leaf-cutter ants. In laboratory and field experiments, we (1) observed a higher survivorship of dispersing *A. fungicola* in an artificial fungal garden chamber compared to *A. fungicola* in artificial brood chambers; (2) evaluated a possible dispersal mechanism, wherein *A. fungicola* ride vectoring alates into non-natal mature *A. texana* colonies; (3) documented a bias of dispersing *A. fungicola* against riding on male alates; and (4) observed for the first time a mature male *A. fungicola* collected from a dispersing *A. texana* female during her mating flight.

The rapid mortality of *A. fungicola* in artificial brood chambers in association with *A. texana* queens suggests that *A. fungicola* unlikely persists through the earliest stages of colony development in the field. This result agrees with the observations of Sánchez-Peña (2005, p. 12), who reported that the maximum survivorship of four *Attaphila* (sp. unreported) individuals placed in artificial brood chambers with *A. mexicana* queens was 15 days. Instead, the high survivorship of *A. fungicola* in a non-natal fungal garden chamber with *A. texana* workers (Fig. 3), and the frequent interactions and apparent tolerance of the workers in the experimental garden toward the cockroaches (see Online Resource 1) suggests that *A. fungicola* may be capable of integrating into established leaf-cutter colonies after mating flights. These results are consistent with the hypothesis that *A. fungicola* use female alates primarily as vectors during the ants' mating flights, but separate from their vectors before or during nest founding to disperse to established colonies. However, our experiment with the artificial fungal garden chamber represents a single trial ($n = 1$, Table 1) 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 our laboratory garden compared

to the expected survivorship under natural conditions in the field.

We found no difference in survivorship between queens with *A. fungicola* absent or present in their brood chambers (Fig. 3), or between the proportion of incipient fungal gardens persisting until the final day of the experiment. This suggests that *A. fungicola* provide no net survival benefits to foundress females or their fungal gardens during the early, worker-less nest-founding stage. Among ants, *Atta* species have some of the highest mortality estimates for foundresses (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, *A. fungicola* that remain with queens during early stages of colony development likely suffer similarly high rates of mortality. We also observed some *Attaphila* touching and grasping the incipient fungal garden of foundresses, which could negatively impact the already fragile condition of their host in early development (Marti et al. 2015; Nehring et al. 2016).

Established leaf-cutter colonies experience lower mortality than incipient colonies (Weber 1972; Fowler et al. 1986; Perfecto and Vandermeer 1993); therefore, the fungal chambers of established colonies may be the preferred destinations of dispersing *A. fungicola*. Moser (1964) has shown that *A. fungicola* follow the chemical trails of *A. texana* under laboratory conditions, suggesting a mechanism for locating established nests if *A. fungicola* dislodges from vectoring female *A. texana* during or after mating flights. However, we are not aware of any direct observations that *A. fungicola* indeed enter established *A. texana* colonies after mating flights, and the fitness costs of locating and integrating into established colonies, compared to the costs of remaining with nest-founding queens, remain to be investigated. In our field experiment, no solitary *A. fungicola* introduced at the margin of a non-natal nest mound after a mating flight entered the nest.

On the other hand, a significant proportion of *A. fungicola* introduced at the margin of a non-natal nest, but attached to their vectoring *A. texana* alates, entered the nest while still attached to these alates. In addition, *A. fungicola* attached to alates introduced into a lab colony detached from alates and dispersed to a fungal garden chamber. Combined, these results suggest a dispersal mechanism heretofore unconsidered in the literature on this myrmecophile: *A. fungicola* ride vectoring female *A. texana* alates during mating flights, remain attached as the vectoring alates are captured or enter established *A. texana* nests, then detach from the alates and move independently to fungal garden chambers. The probability of *A. texana* alates landing in proximity of established colonies and how often this

results in the alates' inclusion into these nests is unknown, making it difficult to assess the costs of this potential *A. fungicola* dispersal strategy.

Due to the natural migration of one of our *Atta* colonies in the field, we collected *A. fungicola* from the same colony at two different nests sites during two separate years of collecting. The frequency of *A. fungicola* collected during both years was similar (approximately 6%). One possible explanation for this apparently stable prevalence is that resident *A. fungicola* migrated with the established colony between nests. If this is the case, the mode of *A. fungicola* movement during an *A. texana* nest migration may inform the strategies *A. fungicola* uses when dispersing to new colonies. Interestingly, this scenario would also suggest that if *A. fungicola* populations have a negative impact on established colonies, nest migration may be an ineffective strategy for *A. texana* colonies to escape the "cockroach infection."

The preference of *A. fungicola* to associate with female alates observed in our lab assays corroborates field observations (Moser 1967; Phillips and Mueller unpublished observations) and may be based on variation in the flight distances of male and female *A. texana* alates; however, the possible variation in dispersal distances between male and female alates has not been thoroughly studied (Moser 1967). Alternatively, if *A. fungicola* remain with newly mated *A. texana* queens through nest founding and subsequent colony development, the bias of *A. fungicola* against male alates may simply be because males represent an evolutionary dead end for *A. fungicola* symbionts.

A better understanding of *Attaphila* reproductive biology (Waller and Moser 1990; Roth 1995), population genetics (Prugnolle and de Meeus 2002), and more frequent and directed sampling of leaf-cutter nests would help to elucidate dispersal strategies of *A. fungicola*. For instance, no male *A. fungicola* have been reported from Louisiana (Moser 1964; Waller and Moser 1990), and this absence could reflect an artifact of limited sampling, or it could indicate parthenogenesis, a reproductive mode that has implications for dispersal (Vrijenhoek and Parker 2009; Verhoeven and Biere 2013). In addition, vector-independent dispersal of *A. fungicola* between leaf-cutter nests remains a possibility (Bryan Ospina, pers. comm.), a mode of dispersal that could be investigated using field assays and sampling around leaf-cutter nests (e.g., year-round sampling with pitfall traps).

Intriguing parallels of dispersal exist between *Attaphila* and *Attacobius*, a genus of spiders including at least two species (*Attacobius luederwaldti* and *A. attarum*) that inhabit the nests of leaf-cutter ants in South America. Like *A. fungicola*, the spiders use leaf-cutter alates as vectors (Platnick and Baptista 1995); during mating flights, *Attacobius* have been observed riding on female leaf-cutter alates at a significantly higher frequency than male alates (Ichinose et al. 2004; Camargo et al. 2015). Furthermore, the proportion (3.1%) of *Attacobius attarum* males collected during a mating flight of *Atta sexdens* (Camargo et al. 2015) is similar to the proportion (1.2%) of *A. fungicola* males we collected during an *A. texana* mating flight, suggesting a similar sex-ratio bias in dispersal. As with *Attaphila*, the destination of *Attacobius* after mating flights remains unresolved (Erthal and Tonhasca 2001; Ichinose et al. 2004; Camargo et al. 2015), but a systematic comparison of cockroach and spider symbionts could reveal general patterns in the evolution of their dispersal strategies, preference for female alates as vectors, and in the female-biased dispersal of the symbionts (Prugnolle and de Meeus 2002; Zepeda-Paulo et al. 2015).

In artificial brood chambers, we paired *A. fungicola* with mated queens that had not acted as vectors for the cockroaches and likely dispersed from different natal colonies. Therefore, this study does not account for interactions before and during mating flights between *A. fungicola* and their host alates that could influence *A. fungicola* survivorship in brood chambers. For instance, *A. fungicola* and female alates dispersing together from the same natal colony may retain similar hydrocarbon profiles, which could later reduce aggressive behavior of mated queens toward *A. fungicola* co-existing in their brood chamber (e.g., the two queen-caused mortalities of *A. fungicola* in this study). Although laboratory pairings of *A. fungicola* and *A. texana* foundresses collected together could better represent natural brood chamber interactions, no *A. fungicola* has been reported attached to a foundress inside her incipient nest.

This understudied ant-cockroach symbiosis may be a fruitful avenue of research to address broader questions in myrmecophile ecology and evolution (Bell et al. 2007). Given the extensive distribution of *Attaphila* in the Americas (Wheeler 1900; Bolívar, 1901, 1905; Brossut 1976; Sánchez-Peña 2005; Rodríguez et al. 2013) and the variation in mating flights and nest-founding strategies among leaf-cutter ants across the Neotropics (Weber 1972; Cahan and Julian 1999; Moser et al. 2004), it is possible that *Attaphila* spp. also express variation in dispersal strategies like their host species. To further elucidate the interactions between this group of cockroaches and their leaf-cutter ant hosts, direct observations of dispersal patterns are needed across its diverse host-species associations and across its entire Neotropical range.

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