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RESEARCH ARTICLE

Foundress queen mortality and early colony growth of the leafcutter ant, *Atta texana* (Formicidae, Hymenoptera)

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Abstract Nest-founding queens of social insects typically experience high mortality rates. Mortality is particularly severe in leafcutter ants of the fungus-growing ant genus *Atta* that face the challenge of cultivating a delicate fungus garden in addition to raising brood. We quantified foundress queen survivorship of *Atta texana* that were collected in northwest Texas and maintained in single-queen laboratory nests, and we tracked the rate of colony growth during the first precarious months of the colony lifecycle. Ninety days post-mating flight, only 16.3 % of 141 of the original queens had survived, and colony growth rates varied markedly across the surviving colonies. Worker production was weakly correlated with fungus garden growth over the course of early colony development. Dead queens became overgrown by the parasitic fungi *Fusarium oxysporum* (26 % of dead queens) and *Aspergillus flavus* (34 %), and these fungi are therefore possible causes of queen mortality. The phorid fly *Megaselia scalaris* emerged from one dead queen, but was unlikely the cause of death. Under natural conditions, intense competition between conspecific colonies can amplify small differences in initial growth rates to generate drastic differences in colony fitness. The observed variation in colony growth rate therefore suggests that colony growth is likely an important target for selection to optimize fitness in *Atta texana*.

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Introduction

The nest-founding stage is a particularly critical stage in the life history of social insects (Oster and Wilson 1978). Nest-founding queens typically experience low survivorship, which creates a selective bottleneck where a very small proportion of surviving queens contribute to the next generation (Brian 1965; Wilson 1971; Cole 2009). Direct observations of foundress survivorship are lacking for most ant species, but for those studies that do exist (e.g., *Pogonomyrmex occidentalis*, *Crematogaster ashmeadi*, *Solenopsis invicta*, *Atta bisphaerica*), the reported percentages of queens surviving to produce incipient colonies range between 0.09 and 7.6 % (Cole 2009 and references therein). Using the Texas leafcutter ant, *Atta texana*, we expand on earlier work by combining new observations on the causes of queen mortality and early colony growth dynamics with previously published information to elucidate trends common across leafcutter ant species (*Atta* and *Acromyrmex*).

Leafcutter ants of the genus *Atta* have some of the lowest estimates of foundress survivorship among ants (Jacoby 1944; Autuori 1950; Fowler 1987; Cole 2009). Direct observation of foundress survivorship in *Atta bisphaerica* estimated only 0.09 % of queens surviving the nest-founding stage (Fowler 1987). Low survivorship in *Atta* and other fungus-growing ants is thought to be due to the compounded challenges of cultivating a delicate fungus garden while raising the first worker-brood, avoiding predators, resisting execution by conspecifics, and coping with pathogens and

parasites (Weber 1972; Fowler et al. 1984, 1986; Fowler 1987).

Of colonies that do survive the nest-founding stage in claustral *Atta* spp. in the field, the first workers open the sealed foundress-nest approximately 1.5–3 months after founding (Huber 1905; Autuori 1942; Moser 1967; Weber 1972). In the laboratory, measures of foundress survivorship exist for *A. texana*, including comparison of survivorship in multiple-queen versus single-queen nests (Mintzer and Vinson 1985; Mintzer 1987). Survivorship is higher in the lab than the field; and fitness is higher for multiple-queen than single-queens nests, as measured by survivorship, worker number and fungus garden size (Mintzer and Vinson 1985; Mintzer 1987). Nests founded by multiple queens occur at a low rate across the range of *A. texana,* 13 % for a population in central Texas (Mintzer and Vinson 1985), and 2.5 % nests in the population studied here at the range limit in northwest Texas (Mueller unpublished observation).

While colony growth is typically measured by the number of workers, the size of the fungus garden is also a key component of colony growth for attine ants, because stored energy resources of a founding queen are allocated to both the production of workers and the cultivation of the incipient fungus garden (Cahan and Julian 1999; Seal and Tschinkel 2007; Clark and Fewell 2014). Previous studies on the semi-claustral, attine species *Acromyrmex versicolor* (Cahan and Julian 1999; Clark and Fewell 2014) and *Trachymyrmex septentrionalis* (Seal and Tschinkel 2007) suggest that the growth relationship between fungus garden size and worker number is variable over the first few months of colony growth and stabilizes sometime before colonies reach sexual maturity. The relationship between the early growth rates of worker number and garden size are unknown for *Atta*. To explore the early growth dynamics in *Atta texana* colonies, we quantified foundress queen survivorship, tracked the rate of colony growth of surviving colonies during the first 3 months after nest founding, and compared growth rates of worker number and fungus garden size.

The causes of death for *A. texana* queens are not well known. Past studies of other ant species suggested that exposure to parasites and pathogens during the mating flight could be significant causes of death (Fernández-Marín et al. 2004; Augustin et al. 2011). Furthermore, without workers to perform social immune behaviors, such as allogrooming, solitary founding queens are especially susceptible to infection (Ho and Frederickson 2014). *Escovopsis* spp. are the most commonly studied fungal parasites of gardens, which can infect the gardens of many tropical fungus-growing ants (Currie et al. 1999; Meirelles et al. 2015). *Escovopsis* was not found to infect gardens of *A. texana* in central Texas (Rodrigues et al. 2011a), and has only been found so far in a few *A. texana* gardens in south Texas (Mueller, unpublished observation). Other parasitic fungi are more frequently found in *A. texana* gardens, including *Syncephalastrum racemosum*, *Fusarium oxysporum*, *Aspergillus flavus,* and *Acremonium polychromum* (Rodrigues et al. 2011a; Seal and Mueller 2014). Ecologically similar fungi, have been isolated on newly mated *Atta capiguara* and *Atta laevigata* queens in Brazil, such as *Acremonium* spp, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium graminearum*, *Paecilomyces lilacinus*, *Trichoderma atroviride*, and *Beauveria bassiana* (Rodrigues et al. 2011b).

Phorid fly parasitoids of several genera are also a source of mortality in leafcutter ants, including *Myrmosicarius* (Borgmeier 1928), *Procliniella* and *Stenoneurellys* (Borgmeier 1931), *Apocephalus* (Brown 1997), *Eibesfeldtphora* and *Lucianaphora* (Disney et al. 2008), and *Neodohrniphora* (Disney et al. 2009). These parasitoids lay their eggs in the bodies of ant-hosts, which then pupate in and emerge from the ants' bodies (Porter et al. 1995; Brown 1997). During our study of mortality of incipient *A. texana* colonies, we examined two types of parasitic fungi and a phorid fly as possible causes of death of *A. texana* queens.

Methods

Ant collection and rearing

Atta texana queens searching for nest sites or actively digging nests were collected from three sites in northwest Texas within 2 h following their mating flights. Queens were collected on May 25th 2014 in Glen Rose, TX (N32.24899° W97.73760, elev. 194 m) between 6:30 and 8:00 am ($n = 14$ queens); and on May 26th 2014 in Newcastle, TX (N33.19405° W98.73891, elev. 351 m) between 6:00 and 7:00 am ($n = 60$) and in Fort Belknap, TX (N33.15118° W98.74026, elev. 358 m) between 7:00 and 8:00 am ($n = 67$). Newcastle and Fort Belknap mark the northwestern range limit of *A. texana* (Mueller et al. 2011a, b). Mating flights occurred during early dawn on days following heavy rainfall (e.g., alate reproductives departed 5:44–5:56 am from a nest at Fort Belknap). Within 15–30 min after departure from their nest, the first queens (presumably having mated) were attracted to bright streetlights, where they were collected and transported to the University of Texas at Austin.

Queens were collected into sterile vials in the field, and within 4 h queens were transferred into two types of cylindrical containers, large containers (4 cm × 5.5 cm diameter; $n = 78$) and small containers (5.5 cm × 2 cm diameter; $n = 63$), which were filled with moistened dental plaster to within 1–1.5 cm of the top. The forceps used to transfer queens from collection vials to plaster containers were sterilized for each queen. To reduce the introduction or

cross-contamination of microbes, all containers had tight lids that permitted limited gas exchange.

Queens were kept at 22–24 °C in a room without specific light or humidity regulation; however, humidity in nest containers was always near 100 % due to the moistened plaster. Queens were checked for mortality 9, 12, 45, 70, and 90 days following collection. Queens that did not initiate a garden or lost their fungus gardens were scored as functionally dead, as garden-less queens are unlikely to survive in the field.

Once workers began to pupate (about 45 days after the mating flight), colonies were moved to nest boxes with two 7.5 × 7.5 cm plastic chambers connected by rubber tubing (Mehdiabadi et al. 2006). Ants and fungus gardens were transferred with ethanol-sterilized forceps. The bottom of one of the two connected chambers was lined with moistened dental plaster to generate a humidified nesting chamber, while the other chamber was left empty as a relatively dry foraging chamber. Plaster chambers were kept covered after pouring the plaster to reduce the amount of introduced microbes. The plaster was re-moistened once every week with deionized water. Once a colony had at least 20 workers (about 20 days after emergence of the first worker and about 70–75 days after nest initiation), each colony was fed twice weekly with approximately 100 mg of minced orange pith.

To estimate colony growth rates, we counted the number of workers in each colony and measured the volume of each fungus garden. Because workers were moving, we counted the workers three times and calculated the average of these counts. If the range of the first three counts was greater than seven workers, we counted an additional three times and averaged across all six counts. Additional counts were typically needed when colony size exceeded 60–70 workers. Workers were counted twice per week during the month of July (50–66 days post-mating flight) and once per week for the month of August (70–90 days post-mating flight).

To track the growth of fungus gardens, we measured the volume of each garden once per week beginning on August 4th (70 days post-mating flight), using a method modified from Seal et al. (2014). We estimated the volume of each garden by overlaying a 5-mm grid on the top of each nest box and counting how many grid squares were filled more than 50 % with fungus garden. We also took a measurement of the maximum height of the garden using the same grid squares against the side of each nest box, but measuring the height to the nearest ¼ of a grid square. Because the height of the gardens was approximately uniform, the grid-square estimate and height were multiplied to estimate total garden volume. It was not possible to measure fungus garden volume blindly without awareness of the approximate worker number, because no second naïve experimenter was available. Using the program R 3.1.1 (R Core Team 2013), we tested the relationship between fungus garden volume and worker number at days 70 and 90 using linear regressions.

Fungal isolation and DNA sequencing

Of 141 queens collected, 50 died within the first 2 weeks and were discarded without scoring possible causes of mortality, but we noticed that many of these dead queens had become overgrown with fungi, so the remaining 91 queens were carefully monitored for the emergence of parasitic fungi. For each fungal morphotype visible on these queens, we chose ten queens with the greatest spore and mycelium coverage for isolation. Isolations were performed July 11th, 7 weeks after the initial collection date. Fungal tissue or spores from the lower bodies of dead specimens were streaked on potato-dextrose agar (PDA) plates (DIFCO; Becton, Dickinson and Company; Sparks, MD 21152 USA). Fungi grew on PDA for 1 week at room temperature, at which point the fastest-growing strain of each morphotype was chosen for sequencing. A 1–2 mm piece of tissue was removed with a sterile scalpel and transferred to a 10 % Chelex solution, and DNA was extracted using a basic Chelex protocol (Sigma-Aldrich, St. Louis, MO 63103 USA). The intergenic-spacer (ITS) region of rDNA was amplified for two, morphologically distinct samples using the primer pair ITS4/ITS5, then sequenced on an ABI 3100 Automated Sequencer (Applied Biosystems), following the methods of Rodrigues et al. (2011b). Raw sequence information was edited and assembled in Geneious v 6.0.3. Sequences are deposited at NCBI Genbank under accessions KM284800 and KM284801.

Results and discussion

Colony survivorship

By August 24th, 90 days post-mating flight, 23 of the 141 original queens had survived, for a survivorship of 16.3 % (Fig. 1). When examined by collection site, the percentages of queens surviving after the first 3 months were 0 % (Glen Rose, TX; $n = 14$), 16.7 % (Newcastle TX; $n = 60$), and 19.4 % (Fort Belknap TX; $n = 67$). The highest mortality rates occurred within the first 9 days after the mating flight, and survivorship did not stabilize until the third month of colony establishment (Fig. 1). Survivorship rates observed here in the lab are higher than those reported from field studies for other *Atta* species (0.09–6.6 %; Jacoby 1944; Autuori 1950; Fowler et al. 1986; Cole 2009). Unlike our lab colonies, *Atta* spp. nests in the field are presumably exposed to additional pressures such as predation, execution by neighboring *Atta* spp. nests, desiccation of fungus gardens, and diseases present in natural soil.

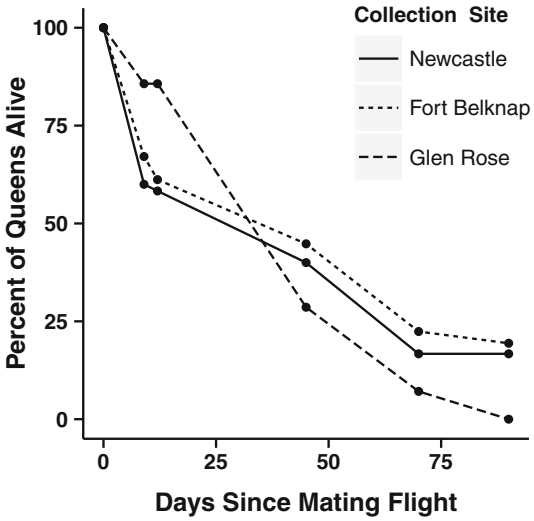

Fig. 1 Survivorship rates of *Atta texana* foundresses collected on the 25th (Glen Rose, TX, $n = 14$) and 26th (Newcastle, TX, $n = 60$; Fort Belknap, TX, $n = 67$) of May 2014

Colony growth

The first workers began to eclose on days 50–55 after nest initiation, which was somewhat later than previously reported for this species. Mintzer and Vinson (1985) and Mintzer (1987) reported worker eclosure after 36–40 days, and 32–42 days, respectively, for laboratory colonies. In field colonies, Moser (1967) estimated first worker eclosure at 40–50 days. It is likely that brood developed slower in our colonies because they were kept at lower temperatures (22–24 °C) compared to those of Mintzer (1987; 27 °C).

Colony growth rates varied markedly between day 56 and day 90, with the fastest colonies outgrowing the slowest colonies by two orders of magnitude (Fig. 2a). Growth differences could be due to differences in stored energy between queens, genotypic differences of the fungal cultivar sustaining colony growth, and differences in associated microbiomes that could be beneficial or detrimental to colony function. Fast-growing leafcutter colonies in the field are less prone to raids by neighboring colonies (Rissing et al. 1989), and in *A. texana*, fast-growing colonies should be able to expand the nest faster towards greater depth and then move the incipient garden to more stable temperature and humidity conditions. Initial colony growth rate, dependent on queen factors (capacity to lay eggs and nourish both brood and garden) and on brood development rate (a function of temperature in the top soil and depth of foundress chamber), therefore may be one of the most important components of colony fitness in *A. texana*.

The growth rates of the fungus gardens also varied between colonies (Fig. 2b). While most gardens increased in volume between days 70 and 90, several remained unchanged or lost volume. Reductions in garden volume occurred most frequently before a colony lost its garden completely.

The relationship between worker production and fungus garden growth was variable during the first 3 months of growth. At day 70, there was a significant, positive relationship between fungus volume and worker number using a linear regression (adjusted $r^2 = 0.456$; $df = 21$; $p = 0.0002$; Fig. 2c); whereas, at day 90 there was no statistically significant relationship between these two growth variables (adjusted $r^2 = 0.009$; $df = 21$; $p = 0.286$; Fig. 2d). This suggests a changing relationship between worker production and fungus garden growth in incipient colonies in *A. texana*. Such changing relationships were found in studies of *Acromyrmex versicolor* and *Trachymyrmex septentrionalis*, but at different stages of development (Cahan and Julian 1999; Seal and Tschinkel 2007; Clark and Fewell 2014). For *Acromyrmex versicolor*, Clark and Fewell (2014) found a developmental transition point at week 27 and colony size of 89 ± 9 workers, when the relationship between worker number and fungus garden growth rates switched from a weak, positive relationship to a strong and stable, positive relationship. Seal and Tschinkel (2007) found a similar pattern in *Trachymyrmex septentrionalis,* in which sexually mature colonies have a much stronger, positive relationship between worker production and garden growth than smaller, incipient colonies. Given the brief, 14-week time period over which we measured growth in our study, it is possible that a similar transition occurs in *A. texana* at a later stage. Overall, there appears to be a trend across these studies that the growth relationship between worker production and fungus garden growth is weak and variable during very early colony development. It is currently unknown if this growth relationship stabilizes in *Atta* colonies as it does in *Acromyrmex* and *Trachymyrmex* colonies at later stages of colony development.

Fungal disease and mortality in *A. texana* queens

Of the 91 queens that we monitored for parasitic fungi, 68 queens died by the end of our study. We could visually distinguish two dominant fungal morphotypes growing on 55 of the 68 dead queens, a white morphotype and a yellow-green morphotype. Twenty-four of the queens were covered in clumps of powdery yellow-green spores; 31 were covered in a dense, white mat of mycelium. We kept collection containers closed until workers began to pupate (about 45 days after mating flight), which prevented cross-contamination by pathogens. It is therefore likely that queens became infected with these pathogens in the field, either prior to the mating flight, during the flight, or when aggregating for nest excavation at our collection sites.

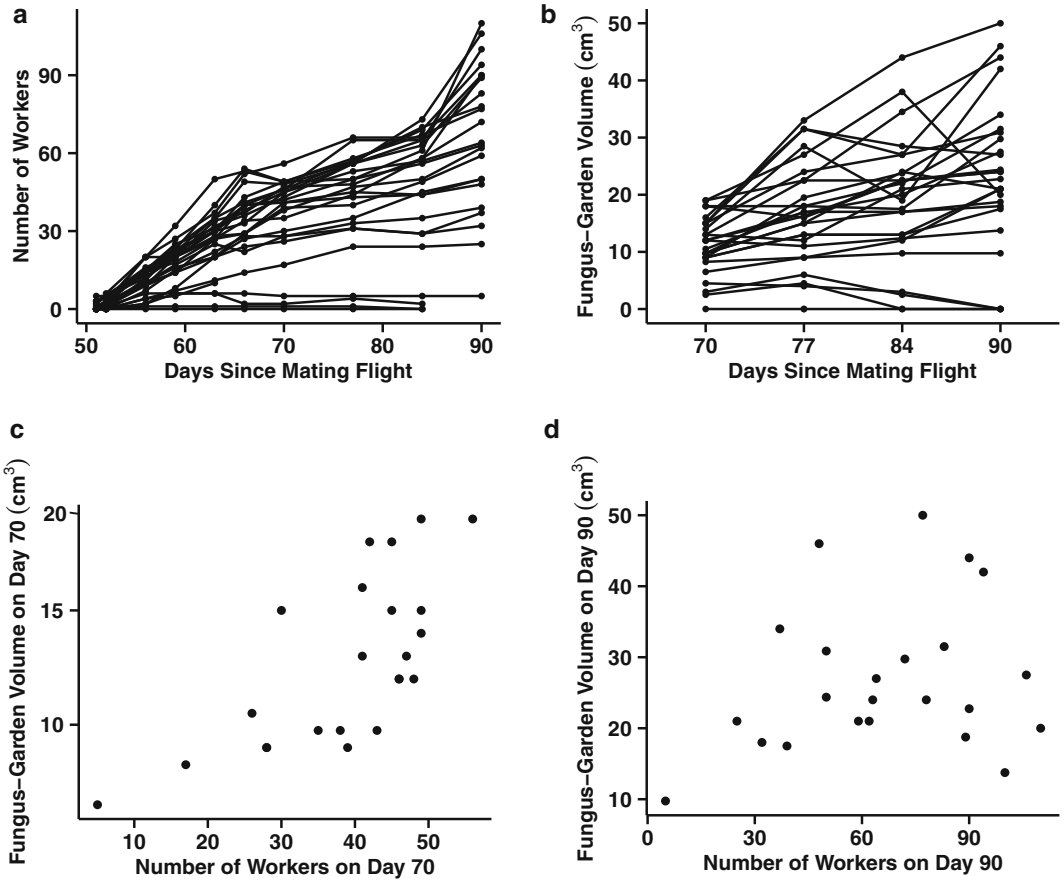

Fig. 2 **a** Colony growth rates as measured by worker number of *Atta texana* colonies between days 56 and 90 post-mating flight. Each of 28 colonies is represented by a *black line*. A line that terminates before day 90 represents a colony that died. **b** Colony growth rates as measured by fungus garden volume between days 70 and 90. **c**–**d** Correlations between worker number and fungus garden volume at days 70 (adjusted $r^2 = 0.456$; $df = 21$; $p = 0.0002$) and 90 (adjusted $r^2 = 0.009$; $df = 21$; $p = 0.286$)

All 20 fungal-isolation attempts were successful and isolates appeared free of contaminants because: (1) each isolation attempt yielded only one morphotype per inoculate; and because (2) there was no visible difference between the replicated isolates for each of the two dominant types (i.e., the fungus appearing white on a queen always yielded a white mycelium on plates; the spore-bearing, yellow-green fungus on queens always yielded a spore-bearing, yellow-green mycelium). Cultures were morphologically highly similar to the two types visible on the queens, suggesting our isolates represent the main fungal morphotypes seen on the dead queens.

DNA sequencing of the ITS gene from one of each of the two morphotypes, identified the two fungi from the dead queens as *Aspergillus flavus* (yellow-green growth) and *Fusarium oxysporum* (white growth). Each of these groups include many species and varieties that cannot be differentiated by the ITS gene. However, in both cases sequence similarity was greater than 98 % to respective sequences deposited in Genbank (http://www.ncbi.nlm.nih.gov) and the molecular species identifications were consistent with the observed culture morphology. *Aspergillus flavus* is a pathogen of animals and plants and is a known, sporadic disease of leafcutter ants (Boucias and Pendland 1998; St. Leger et al. 2000; Hughes and Boomsma 2004). *A. flavus* has also been found in the dump piles (expended garden and dead ants) of several leafcutter species, including *A. colombica*, *Acromyrmex echinatior*, and *Ac. octospinosus* (Hughes unpublished, cited in Hughes and Boomsma 2004). Similarly, *F. oxysporum* has frequently been isolated from *Atta* gardens (Rodrigues et al. 2005, 2008). Rodrigues et al. (2010) isolated *F. oxysporum* from dead queens of *Atta laevigata* and *Atta capiguara* in Brazil, suggesting that perhaps this fungus has the potential to cause disease, but it remained unclear whether the death of the queens was specifically due to *F. oxysporum*. Several species of *Fusarium* can infect a diverse range of insects (O'Donnell et al. 2012), and there is no evidence to date that any *F. oxysporum* lineages are primarily or exclusively ant-associated. *A. flavus* and *F. oxysporum* appear to be pathogens that are harmful to the ants, rather than solely to the garden,

because queens that became overgrown with these fungi died within the first 9 days after their mating flights, which was before they established their gardens. It is possible that these fungus-overgrown queens were energetically weakened during their mating flights, such that opportunistic pathogens were able to overwhelm their immune systems in the following days. A more comprehensive investigation of the foundresses' microbial ecology is needed to assign definitive cause of foundress death to either *A. flavus* or *F. oxysporum*.

Observation of *Megaselia scalaris* feeding on *A. texana*

Megaselia scalaris phorid flies (Phoridae, Diptera) were found with a dead *A. texana* queen on July 9th in its collection container. This queen was collected from Newcastle, Texas, which is the northwestern range limit of *A. texana*. *M. scalaris* feeds on a wide range of decaying organic materials and was likely not the cause of death for this queen, but rather fed on the queen post-mortem (Disney 2008). We can rule out the possibility that *M. scalaris* parasitized this queen, because *M. scalaris* females do not possess the necessary piercing ovipositor to deposit eggs inside a host's body. *M. scalaris* eggs could have been laid on the queen before she was collected; alternatively, it is possible that *M. scalaris* entered the container through an undetected crack in the container housing the queen in the lab, as *M. scalaris* has "an extraordinary capacity to penetrate into or escape from seemingly closed containers" (Disney 2008). *M. scalaris* has not previously been found to feed on *Atta* corpses, but has been found feeding on refuse piles of other ant genera (*Eciton*) and moribund termite alates, along with many other sources of decaying organic material (Disney 2008).

Conclusion

The first 3 months of the *Atta* colony life cycle are particularly fraught with obstacles for foundress queens and their incipient colonies (Weber 1972; Fowler et al. 1984, 1986). As in other ant species (Cole 2009 and references therein), high risk of mortality during nest-founding and intense competition between conspecific colonies can amplify small differences in initial growth rates to have drastic effects on colony fitness. Early growth rate is especially important in determining which colonies will be able to (1) resist execution by other *Atta* colonies (Fowler et al. 1984; Fowler 1987); (2) maintain careful hygiene to cope with diseases of leaf-cutter ants (Yek and Mueller 2011) and of the fungal garden (Mueller et al. 2010); and (3) expand the colony rapidly to reach a stable microclimate at deeper soil layers (Mueller et al. 2011a, b). The observed variation in colony growth rate (Fig. 2) combined with the many obstacles faced by new, small colonies, suggests that early growth rate is likely an important selective factor affecting fitness in *Atta texana*.

Two potential fungal pathogens, *Aspergillus flavus* and *Fusarium oxysporum* infected queens that died before they were able to establish their gardens, suggesting that such pathogens could have detrimental effects on queens during nest establishment. It remains unclear whether the pathogens identified here were the primary cause of death. Experimental exposure of queens to fungal pathogens like *A. flavus* and *F. oxysporum* can determine their virulence and fitness-effects on *A. texana*.

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References

Augustin JO, Santos JFL, Elliot SL (2011) A behavioral repertoire of *Atta sexdens* (Hymenoptera, Formicidae) queens during the claustral founding and ergonomic stages. Insect Soc 58:197–206

Autuori M (1942) Contribuição para o conhecimento da saúva (*Atta* spp. -Hymenoptera -Formicidae) II. O saúveria inicial (*Atta sexdens rubropilosa* Forel, 1908). Arq Inst Biol 13:67–86

Autuori M (1950) Contribuição para o conhecimento da saúva (*Atta* spp. -Hymenoptera -Formicidae) V. Numero de formas aladas e redução dos saúverios iniciais (*Atta sexdens rubropilosa* Forel, 1908). Arq Inst Biol 19:325–331

Borgmeier T (1928) Nota previa sobre alguns phorideos que parasitam formigas cortadeiras dos gêneros *Atta* e *Acromyrmex*. Bot Biol 14:119–126

Borgmeier T (1931) Sobre alguns phorideos que parasitam a saúva e outras formigas cortadeiras (Diptera: Phoridae). Arq Inst Biol 4:209–228

Boucias DG, Pendland JC (1998) Principles of insect pathology. Kluwer Academic Publishers, Dordrecht

Brian MV (1965) Social insect populations. Academic Press, New York

Brown BV (1997) Revision of the *Apocephalus attophilus* group of ant-decapitating flies (Diptera: Phoridae). Contr Sci 468:1–60

Cahan S, Julian GE (1999) Fitness consequences of cooperative colony founding in the desert leaf-cutter ant *Acromyrmex versicolor*. Behav Ecol 10:585–591

Clark RM, Fewell JH (2014) Transitioning from unstable to stable colony growth in the desert leafcutter ant *Acromyrmex versicolor*. Behav Ecol Sociobiol 68:163–171

Cole BJ (2009) The ecological setting of social evolution: The demography of ant populations. In: Gadau J, Fewell JH (eds) Organization of insect societies: from genome to sociocomplexity. Harvard University Press, Cambridge, pp 74–104

Currie CR, Mueller UG, Malloch D (1999) The agricultural pathology of ant fungus gardens. Proc Natl Acad Sci 96:7998–8002

Disney RHL (2008) Natural history of the scuttle fly, *Megaselia scalaris*. Annu Rev Entomol 53:39–60

Disney RHL, Elizalde L, Folgarait PJ (2008) New species and records of scuttle flies (Diptera: Phoridae) associated with leaf-cutter ants and army ants (Hymenoptera: Formicidae) in Argentina. Sociobiology 51:95–117

Disney RHL, Elizalde L, Folgarait PJ (2009) New species and new records of scuttle flies (Diptera: Phoridae) that parasitize leaf-cutter and army ants (Hymenoptera: Formicidae). Sociobiol 54:601–632

Fernández-Marín H, Zimmerman JK, Wcislo WT (2004) Ecological traits and evolutionary sequence of nest establishment in fungus-growing ants (Hymenoptera, Formicidae, Attini). Biol J Linnean Soc 81:39–48

Fowler HG (1987) Colonization patterns of the leaf-cutting ant, *Atta bisphaerica* Forel: evidence for population regulation. J Appl Ent 104:102–105

Fowler HG, Robinson SW, Diehl J (1984) Effect of mature colony density on colonization and initial colony survivorship in *Atta capiguara,* a leaf-cutting ant. Biotropica 16:51–54

Fowler HG, Pereira-da-Silva V, Forti LC, Saes NB (1986) Population dynamics of leaf-cutting ants: A brief review. In: Lofgren CS, Vander Meer RK (eds) Fire ants and leaf-cutting ants: biology and management (studies in insect biology). Westview Press, Boulder, pp 123–145

Ho EKH, Frederickson ME (2014) Alate susceptibility in ants. Ecol Evol 4:4209–4219

Hughes WOH, Boomsma JJ (2004) Let your enemy do the work: within-host interactions between two fungal parasites of leaf-cutting ants. Proc R Soc Lond B (Suppl) 3:S104–S106

Jacoby M (1944) Observações e experiências sôbre *Atta sexdens rubropilosa* Forel visando facilitar seu combate. Bol Min Agric Rio 12:1–55

Mehdiabadi NJ, Hughes B, Mueller UG (2006) Cooperation, conflict, and coevolution in the attine ant-fungus symbiosis. Behav Ecol 17:291–296

Meirelles LA, Montoya QV, Solomon SE, Rodrigues A (2015) New light on the systematics of fungi associated with attine ant gardens and the description of *Escovopsis kreiselii* sp. nov. PLoS One 10:e011206i7

Mintzer AC (1987) Primary polygyny in the ant *Atta texana*: number and weight of females and colony foundation success in the laboratory. Insect Soc 34:108–117

Mintzer A, Vinson SB (1985) Cooperative colony foundation by females of the leafcutting ant *Atta texana* in the laboratory. J New York Entomol Soc 93:1047–1051

Moser JC (1967) Mating Activities of *Atta texana* (Hymenoptera, Formicidae). Insect Soc 14:295–312

Mueller UG, Scott JJ, Ishak HD, Cooper M, Rodrigues A (2010) Monoculture in leafcutter ant gardens. PLoS ONE 5:e12668

Mueller UG, Mikheyev AS, Solomon SE, Cooper M (2011a) Frontier mutualism: co-evolutionary patterns at the northern range limit of the leafcutter ant-fungus symbiosis. Proc R Soc Lond 278:3050–3059

Mueller UG, Mikheyev AS, Hong E, Sen R, Warren DL, Solomon SE, Ishak HD, Cooper M, Miller JL, Shaffer KA, Juenger TE (2011b) Evolution of cold-tolerant fungal symbionts permits winter fungiculture by leafcutter ants at the northern frontier of a tropical ant-fungus symbiosis. Proc Natl Acad Sci 108:4053–4056

O'Donnell K, Humber RA, Geiser D, Kang S, Park B, Robert V, Crous PW, Johnston PR, Aoki T, Rooney AP, Rehner SA (2012) Phylogenetic diversity of insectivorous fusaria inferred from multilocus DNA sequence data and their molecular identification via FUSARIUM-ID and *Fusarium* MLST. Mycology 104:427–445

Oster GF, Wilson EO (1978) Caste and ecology in the social insects. Princeton University Press, Princeton

Porter SD, Pesquero MA, Campiolo S, Fowler HG (1995) Growth and development of *Pseudacteon* phorid fly maggots (Diptera: Phoridae) in the heads of *Solenopsis* fire ant workers (Hymenoptera: Formicidae). Environ Entomol 24:475–479

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rehner SA (2012) Phylogenetic diversity of insectivorous fusaria inferred from multilocus DNA sequence data and their molecular identification via FUSARIUM-ID and *Fusarium* MLST. Mycol 104:427–445

Rissing SW, Pollock GB, Higgins MR, Hagen RH, Smith DR (1989) Foraging specialization without relatedness or dominance among co-founding ant queens. Nature 388:420–422

Rodrigues A, Pagnocca FC, Bacci M Jr, Hebling MJA, Bueno OC, Pfenning LH (2005) Variability of non-mutualistic filamentous fungi associated with *Atta sexdens rubropilosa* nests. Folia Microbiol 50:421–442

Rodrigues A, Bacci M, Mueller UG, Ortiz A, Pagnocca FC (2008) Microfungal "weeds" in the leafcutter ant symbiosis. Microb Ecol 56:604–614

Rodrigues A, Silva A, Bacci M, Forti LC, Pagnocca FC (2010) Filamentous fungi found on foundress queens of leaf-cutting ants (Hymenoptera: Formicidae). J Appl Entomol 134:342–345

Rodrigues A, Mueller UG, Ishak HD, Bacci M Jr, Pagnocca FC (2011a) Ecology of microfungal communities in gardens of fungus-growing ants (Hymenoptera: Formicidae): a year-long survey of three species of attine ants in Central Texas. FEMS Microbiol Ecol 78:244–255

Rodrigues A, Silva A, Bacci M Jr, Forti LC, Pagnocca FC (2011b) Filamentous fungi found on foundress queens of the leaf-cutting ants (Hymenoptera: Formicidae). J Appl Entomol 134:342–345

Seal JN, Mueller UG (2014) Instability of novel ant-fungal associations constrains horizontal exchange of fungal symbionts. Evol Ecol 28:157–176

Seal JN, Tschinkel WR (2007) Energetics of newly-mated queens and colony founding in the fungus-gardening ants *Cyphomyrmex rimosus* and *Trachymyrmex septentrionalis* (Hymenoptera: Formicidae). Physiol Entomol 32:8–15

Seal JN, Schiott M, Mueller UG (2014) Ant-fungus species combinations engineer physiological activity of fungus gardens. J Exp Biol 217:2540–2547

St. Leger RJ, Screen SE, Shams-Pirzadeh B (2000) Lack of host specialization in *Aspergillus flavus*. Appl Environ Microbiol 66:320–324

Weber NA (1972) Gardening ants, the attines. The American Philosophical Society, Philadelphia

Wilson EO (1971) The insect societies. Harvard University Press, Cambridge

Yek SH, Mueller UG (2011) The metapleural gland of ants. Biol Rev 91:201–224