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RESEARCH ARTICLE

The best of heavy queens: influence of post-flight weight on queens' survival and productivity in *Acromyrmex subterraneus* (Forel, 1893) (Hymenoptera: Formicidae)

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Abstract

The weight of post-flight ant queens reflects how much stored resources are available to overpass the new colony foundation phase. *Acromyrmex* queens must dig the nest and forage during this period to cultivate the symbiotic fungus for larvae feeding, implying even higher energetic costs. But if the activities of foraging and excavating were excluded from this phase, would it be possible to mitigate the effect of the queen's post-flight weight on its survival and productivity? In this context, we tracked the survival of 1128 queens already classified into Featherweight, Lightweight, Welterweight and Heavyweight until the emergence of the first workers. Also, for 476 queens we registered the productivity, the proportion of queens that regurgitated the symbiotic fungus, of those that transported it to leaf surface, as well as the proportion that presented some pathogenic fungus on their body surface after death. After the 2nd week, 45% of queens had died and the post-flight weight influenced survival chances, with featherweight queens presenting the highest mortality rate but not the lowest productivity. Here we add the queen's post-flight weight, the absence of symbiotic fungus and, less remarkably, the contamination by pathogenic fungus as other variables that should likewise be considered as factors that reduce the success rate of colony foundation. We highlight that the post-flight weight did not influence the productivity, so when the queen survives, the offspring production is not affected, giving queens of different weights the same chance to become successful at colony foundation.

Keywords Mating flight · Colony foundation · Mortality rate · Leaf-cutting ant · Semiclaustral

Introduction

Queens from leaf-cutting ant colonies (*Atta* and *Acromyrmex*) are responsible for colony reproduction and for establishing a new colony (Wilson 1980; Hölldobler and Wilson 1990). They can survive for decades (Pamilo 1991) and to become successful in a new colony foundation they must overcome two great challenges: the nuptial flight (Fjerdingstad and Keller 2004) and the colony foundation phase itself. To surpass these challenges, ant queens have two forms of storing energy; soluble carbohydrates, that are used as fuel during the nuptial flight (Jutsum and Quinlan 1978; Silva et al. 2015), and fat, used during the nest foundation phase to sustain themselves and perform all the colony's activities until the worker caste emergence (Della Lucia et al. 1995; Silva et al. 2015). Another source of energy comes from the flight muscles, whose catabolism starts just after the insemination (Helms and Kaspari 2014, 2015).

A primary factor that subsidizes queens' survival during the nest foundation phase is how much accumulated energy they have, which can be measured through their body mass (Seal 2009). Although larger queens could be more fertile and successful in founding colonies than smaller ones (Mintzer 1987; Vander Meer et al. 1992; Tschinkel 1993; Bernasconi and Keller 1999; Wagner and Gordon 1999; Johnson 2001; Wiernasz and Cole 2003), there is a marginal value for fat accumulation, since a high weight limits flight ability (Vogt et al. 2000; Wiernasz and Cole 2003), natal nest dispersal distance (Sundström 1995; Rüppell et al. 1998;

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Lachaud et al. 1999) and predator evasion (Fjerdingstad and Keller 2004), especially from birds (Mariconi 1970).

The high mortality rate of founder queens is also related to excavation effort (Camargo et al. 2011), being killed by conspecifics (Fowler et al. 1984), desiccation (Peeters and Ito 2001) or contamination by pathogenic fungus (Hughes et al. 2009). The exposure to risks during the foundation phase is thought to be even greater for *Acromyrmex,* because it presents a semi-claustral behavior. Semi-claustral queens must leave the colony in search for food (Peeters and Ito 2001), which could contribute to a higher mortality rate for *Acromyrmex* than that for *Atta,* which has a claustral foundation. *Atta* mortality rate during colony foundation phase is around 95–99.95% in laboratory conditions (Augustin and Lopes 2011). Due to lower fat accumulation, species with semi-claustral foundation, like *Acromyrmex*, allocate the remaining energy after flight and excavation to finding food and transporting leaves into the colony for cultivating the fungus garden (Hölldobler and Wilson 1990; Seal and Tschinkel 2007).

According to the Found or Fly hypothesis (Helms and Kaspari 2014, 2015) the success of nuptial flight and colony founding emerge from an ecological trade-off mediated by the queen's abdomen weight. Queens with heavier abdomens have less maneuverability during flight, and thus would be less able to mate, disperse and evade predators. On the other hand, queens with lighter abdomens would not have enough fat stored to survive and invest in offspring production, especially if they had to dig and forage during the foundation phase. Here, it is suggested that queens of all weights which surpass the challenge of nuptial flight will have the same survival probability and productivity rates if they are deprived of the need of foraging and digging, tasks of high energy expenditure and risk (Fewell et al. 1996; Camargo et al. 2011). Our hypothesis is based on the fact that offspring from semi-claustral queens nutritionally depend on mostly resources foraged by the queen (Seal 2009).

This study assessed the influence of the post-flight weight of *Acromyrmex subterraneus* (Forel, 1893) foundresses on their survival and offspring production during the foundation period after the nuptial flight when exposed to conditions that do not require nest excavation and external foraging. In addition, some behavioral and ecological aspects related to nest foundation and *mortis* cause were reported.

Materials and methods

Study species

Acromyrmex subterraneus is a leaf-cutting ant species which exhibits a symbiotic relationship with *Leucoagaricus gongylophorus* Möller (Singer), the main food source for the colony, which is horizontally transferred within colonies by the founder queens, that carry a fungus pellet inside their infrabuccal cavity (Mintzer 1987). The fat content in genus *Acromyrmex* represents about 30% of their total body weight (Seal 2009).

Queens were collected after the nuptial flight in two consecutive years—2011 and 2012—at the Federal University of Juiz de Fora, Brazil (21° 46′ 43.65″ S, 43° 21′ 16.84″ W). The area is urban, with many facilities and garden areas with ground surface lawn or exposed soil. The nuptial flight occurred between November and early December, always on a muggy, cloudy morning, with high humidity and temperature, after heavy rains on the previous day. Collection took place between 7:30 am and 10:00 am. In total, 1128 queens were captured—476 in 2011 and 652 in 2012, regardless of whether they were winged or not. Soon after capture, each queen was weighed on an analytical precision scale and placed in plastic containers (250 mL) with 1 cm of plaster at the bottom, regularly moistened. The container served as the colony's first chamber and, thus, the queens did not need to excavate. Every two days, a similar amount of fresh leaves of *Acalypha wilkesiana* Müll.Arg. (Euphorbiaceae) was provided inside the colonies, therefore excluding the energy expenditure in foraging. The temperature and humidity in the laboratory were maintained at approximately 25 °C and 80%, and the colonies were covered with red cellophane to reduce light exposure.

Queens' survival

The queens' survival in 2011 and 2012 was checked weekly during the foundation period, that is, until the emergence of the first workers. The weight of each queen was also measured weekly.

Queens' productivity

Productivity was investigated only for queens collected in 2011. The number of eggs of each queen was counted weekly during 13 weeks, thus allowing examination of the oviposition rate variation. Also, the total number of offspring was counted—eggs, larvae, pupae and workers—in the 13th week, thus allowing registering of the population size reached soon after the end of the foundation phase. The week in which worker development forms (egg, larva, pupa, adult) were first observed in each colony was registered, along with how many individuals composed the first generation of workers.

Further relevant information

For the 2011 collection, some characteristics and behaviors shown by the queens were registered. On the collection day, the number of collected winged and unwinged queens was recorded. During the first week of foundation, the proportion of queens that regurgitated the symbiotic fungus and of those that transferred the fungus garden to the provided plant substrate was recorded. In addition, after death, all queens were visually analyzed to look for some type of pathogenic fungus on their body surface, allowing for the attribution of a death percentual to pathogenic fungi. These observations were analyzed descriptively.

Statistical analysis

The survival of the queens was modeled through the Weibull distribution, whose parameters allow greater flexibility to model systems in which the number of failures—queen's death—increases, decreases or remains constant over time. In this case, data are censored (Crawley 2007).

In this experiment, the starting point of the observations was determined as the queen collection day, while the end point was when the queens died (censor 1) or when the first workers emerged (censor 0). Likelihood of survival was considered as a response variable, and the week of death as an explanatory variable. Considering all sampled individuals, post-flight weight variation ranged between 0.0229 and 0.0598 g. Queens' post-flight weight data were divided into four categorical classes based on quartiles. The first quartile comprises of featherweight queens—FW (≤ 0.0472 g), the second, lightweight queens—LW (> 0.0472 g and ≤ 0.0498 g), the third, welterweight ones—WW (> 0.0498 g and ≤ 0.0523 g) and the fourth, heavyweight queens (HW > 0.0523 g). Weight classes were considered a covariate. In total, 680 censored cases (censor 1) and 448 uncensored cases (censor 0) were used. The explanatory model differed significantly from the null model ($p = 0.002$). Survival curves from each weight class were compared through Tukey's test at 5% significance. Also, we submitted data to a Cox proportional-hazards model to verify the association between the survival time of queens of different weight classes.

The queens' productivity during the foundation period was correlated with the post-flight weight by a GLM with Poisson distribution, considering the weight categories. Productivity was considered a response variable, while post-flight weight was an explanatory variable. Also we compared the number of eggs counted weekly among queens' weight classes applying a zero-inflated model, with negative binomial distribution. This model was chosen because of the overdispersion and frequent zero-valued observations (Zuur et al. 2009).

All analyses were done using the R 3.6.0 program (R Core Team 2019) and the packages "AER" (Kleiber and Zeileis 2008), "survival" (Therneau 2015), "multcomp" (Hothorn et al. 2008), "survminer" (Kassambara et al. 2019), "pscl" (Zeileis et al. 2008), "ggsci" (Xiao 2018), "ggplot2" (Wickham 2016) and "ggpubr" (Kassambara 2019).

Results

Considering the 1,128 queens collected in 2011 and 2012, about 45% died in the first week after the nuptial flight. The likelihood of survival increased over time (χ=20.69, $df = 3$, $n = 1{,}128$, $p < 0.001$), characterizing a type-III survival curve, in which mortality is high in early life, with higher chances of survival later.

The survival curve of featherweight queens was lower than those of the other weight classes (FW and LW: $z = 3.772$, $p < 0.001$; FW and WW: $z = 3.520$, $p < 0.001$; FW and HW: $z = 3.759$, $p < 0.001$). In fact, survival probability of featherweight queens was lower than 50% in the 2nd week, while the other weight classes reached this mark around the 9th week (Fig. 1). The death hazard for FW queens is about 1.42 times more than for the queens from the other weight classes.

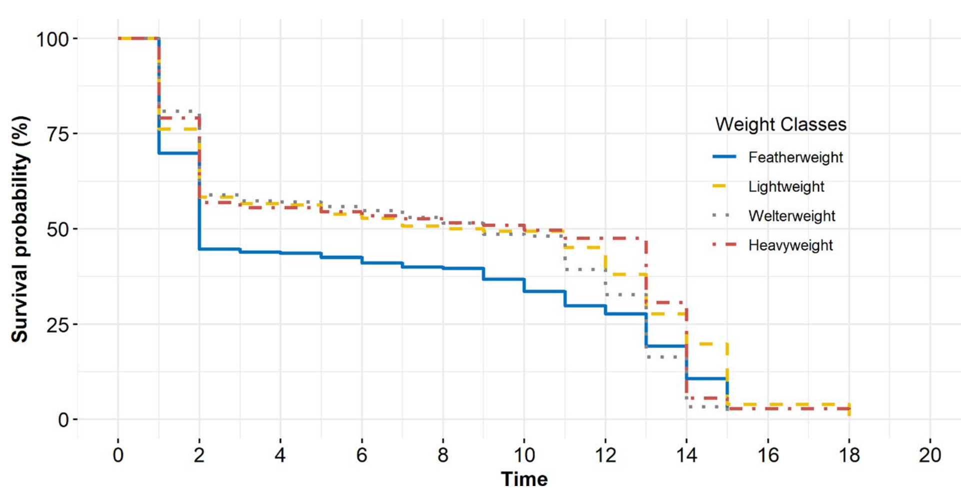

Fig. 1 Survival probability of *Acromyrmex subterraneus* queens during the colony foundation phase, considering post-flight weight as covariate. Weight data were separated into four categories according to median and quartiles. Data collected in 2011 and 2012

On the other hand the queens' post-flight weight does not explain the variation in productivity ($Z = -0.36$, $p = 0.72$) (Fig. 2). Queens from different weight classes were verified with both low and high productivity, which means that post-flight weight did not interfere in productivity.

Out of the 476 queens sampled in 2011, about 53.2% ($n = 253$) regurgitated the symbiotic fungus, while the others, from which the fungus regurgitation was not registered, all died soon in the first week. Fungus regurgitation occurred mainly on the second day after the nuptial flight, and its deposition was usually done directly on the plaster layer or on the wall of the container. From those that regurgitated the fungus we verified that about 8.7% ($n = 22$) had been infected by some type of pathogenic fungus (W.O.H. Hughes personal communication) (Fig. 3) and died before the emergence of the first workers.

Of the approximately 30.25% of queens that survived until the emergence of the first workers ($n = 144$), 12.5% did not remove their wings ($n = 18$) and 41% had transferred the regurgitated fungus ($n = 59$) to the surface of the leaf fragment provided as food substrate. It is interesting

Fig. 2 Relationship between post-flight weight of *Acromyrmex subterraneus* queens and productivity (accumulated number of eggs, larvae, pupae and workers) over 15 weeks. Data collected in 2011

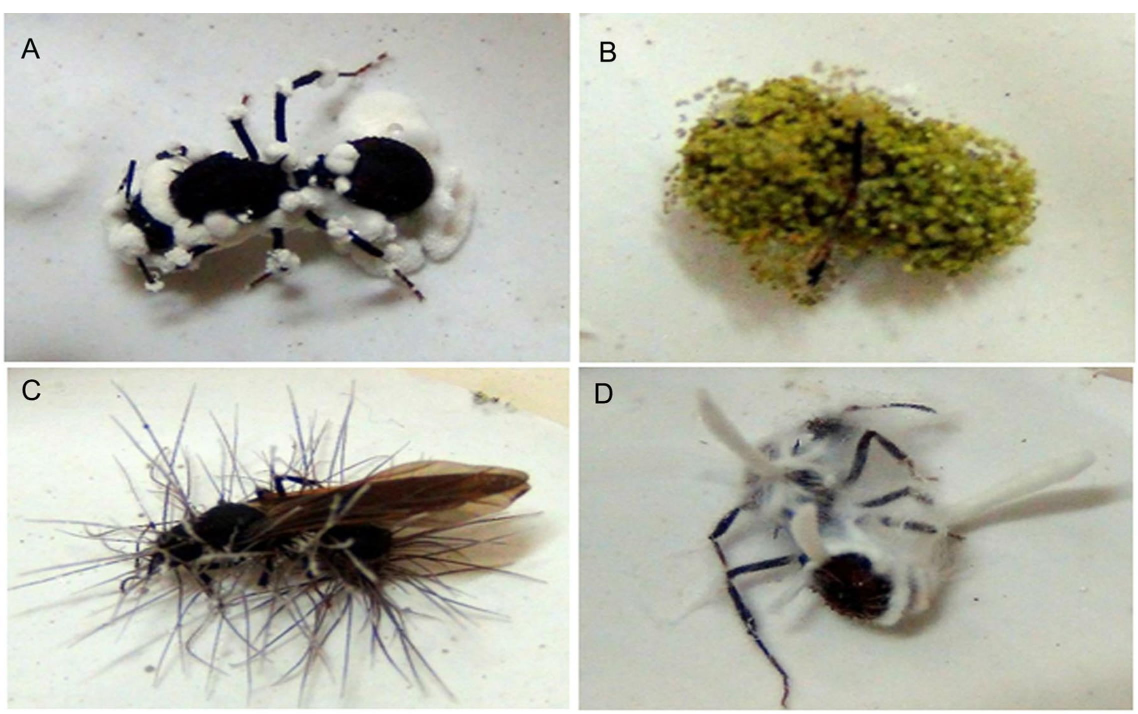

Fig. 3 *Acromyrmex subterraneus* queens infected with entomopathogenic fungi. **a** *Metarhizium anisopliae*; **b** *Aspergillus flavus*; **c** and **d** unidentified

to note that the queens tended to grow the fungus garden in the shape of a pot, keeping the offspring inside.

Eggs were laid soon in the 1st week after the nuptial flight. Larvae appeared in the 3rd week, pupae between the 6th and 7th weeks. In 49.6% of the colonies, the first generation of workers appeared in the 9th week after the nuptial flight. The first generation was composed on average of two workers, on the 13th week the colonies with the largest population ($n = 5$) had 24 workers. The number of eggs per week did not differ among queens' weight classes, and grew cumulatively throughout the weeks (Fig. 4).

Discussion

For *Ac. subterraneus*, the survival of queens along the weeks varied as a function of their post-flight weight, with the featherweight queens displaying less survival probability. Even excluding the high-risk factors such as excavation and foraging that favor mortality, the likelihood of survival was still low during the colony foundation phase, especially in the first two weeks. The mortality rate of *Ac. subterraneus* reached the highest value (45%) within the 1st and 2nd weeks, similar to that of *Acromyrmex octospinosus* (Reich, 1793) (48%) in field colonies along the first month after the nuptial flight (Fernández-Marín et al. 2003).

In *Atta sexdens* colonies raised in laboratories under the same conditions of this study, queens' mortality reached about 58.3% within up to one week after the nuptial flight (Augustin and Lopes 2011). In *Atta texana*, the highest mortality rate is found within the first nine days after the nuptial flight and, in the same way that we registered for *Ac. subterraneus*, the survival probability did not stabilize until the third month of colony foundation (Marti et al. 2015), which coincides with the end of this phase.

Although *Atta* and *Acromyrmex* have different types of foundation—claustral and semi-claustral, they nonetheless present high mortality rates. This is related to both species sharing an independent nest founding strategy (Fernández-Marín et al. 2004). Species that present this strategy release thousands of reproductive individuals (Hölldobler and Wilson 1990) and, no matter if they close themselves inside the nest or sometimes leave to forage during the foundation phase, they experience high risk of death from the moment they leave the mother colony until the beginning of the ergonomic phase (Schmid-Hempel 1984; Hölldobler and Wilson 1990; Peeters and Ito 2001).

Commonly, the high mortality of queens during and after nuptial flight is associated with predation by birds, lizards and other ant species (Fowler et al. 1984; Robertson and Villet 1989; Hölldobler and Wilson 1990; Marti et al. 2015), drowning or desiccation during the flight (Peeters and Ito 2001), excavation effort (Camargo et al. 2011), or even contamination by pathogenic fungus during excavation or foraging (Hughes et al. 2009; Augustin et al. 2013; Marti et al. 2015). All of these risk factors after flight were excluded in this study, as it was conducted in a laboratory and queens were provided with food and an artificial nest. Instead, chances of survival at the end of 15 weeks for *Ac. subterraneus* were smaller than 0.1%, indicating that the causes of this high mortality go beyond the previously mentioned factors. The lower likelihood of survival of featherweight queens, coupled with the suppression of excavation and food search activities, is indicative that the queen's weight itself is a relevant factor that influences the mortality rate.

Surprisingly, queens' productivity was similar for the different weight classes, meaning that the post-flight weight did not influence the queens' productivity. The statement that heavier queens invest all the extra weight into offspring production and so produce more offspring and faster during the founding period (Wiernasz and Cole 2003; Helms and Kaspari 2014; Marti et al. 2015; Moreira et al. 2019) was not confirmed for *Ac. subterraneus*.

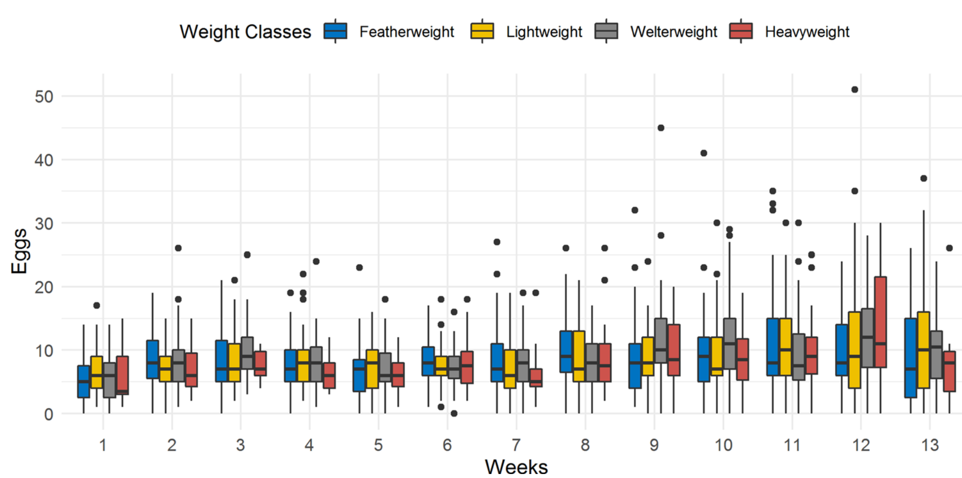

Fig. 4 Number of eggs produced by queens from different post-flight weight classes along 13 weeks. Box-and-whisker plot showing median (horizontal line), interquartile range (box), distance from upper and lower quartiles (whiskers), outliers (> 1.5 × upper or lower quartile). Data collected in 2011

The fact that productivity did not differ between queens of different weights may be associated with the energy savings promoted by the suppression of excavation and food searching activities. Furthermore, in several semi-claustral ant species the queen uses the foraged food to nourish the offspring, instead of their own stocked energy (Seal 2009). Consequently, featherweight queens may have been privileged by not having to do the suppressed activities, thus reaching the ergonomic phase with similar offspring production to the other queens' weight classes. Also, queens of all weight classes received the same amount of leaves, thus having the opportunity to obtain the same amount of energy to convert into offspring.

Other observed events may be related to the low recorded survival rate. It is suggested that the high mortality rate is also associated with the absence of the symbiotic fungus, since all queens that did not regurgitate the symbiotic fungus died in the first week, these represented about 47% of collected queens. Species from Attini tribe have an obligatory symbiotic relationship with fungi of families Lepiotaceae and Pterulaceae (Basidiomycota) (Schultz and Brady 2008; Augustin et al. 2013), which are the colony's main food source. This relationship is so solid that, upon leaving the mother colony, the virgin queens must carry in their infrabuccal cavity a fungus pellet that will be the starting crop for their new garden. Leaving from the mother colony without the symbiotic fungus, or losing it, is a common event for Attini queens (Mueller et al. 1998; Fernández-Marín et al. 2004). *Acromyrmex* queens can accept new fungus gardens and survive what is an indication that the symbiotic fungus is easily lost (Bot et al. 2001). In both cases, there are alternative actions performed by queens that can repair the fatality of the absence of the symbiotic fungus such as: joining an already established colony that has the fungus garden; recruiting other founders that have the fungus pellet (co-foundation); stealing the fungus from another nest, or also finding an external source of compatible fungus (Fernández-Marín et al. 2004). In this study, however, the queens did not have any of these possibilities, so the fungus' absence was consistent with their death cause.

Deposition of fungus on the side of the fungus chamber container and its transfer to the leaf surface (provided as fungus substrate) may be actions related to hygienic behavior. Depositing fungus on root platforms or not directly on the floor is a behavior already reported for Attini queens (Fernández-Marín et al. 2004, 2007). These actions, together with self-grooming and frequent leg-cleaning prior to substrate manipulation, may allow the fungus garden to grow free of pathogens, which may be an adaptation to boost survival (Fernández-Marín et al. 2003; Augustin and Lopes 2011). *Ac. octospinosus* queen founders, for instance, apparently do not select plant species to attach the fungus. However, in the absence of a root in the primary chamber, the pellet can be attached to a stone or to the ceiling of the chamber (Fernández-Marín et al. 2004).

Some queens that did not remove their wings succeeded the nest foundation phase and were able to produce offspring. Although present in all of the weight classes, their number ($n = 19$) was too small to allow a comparison between their productivity with that of queens that removed their wings. Despite a lack of statistical comparison, it seems that the role of flight muscles as fuel for colony growth is secondary, as winged queens also founded their colonies. Even for claustral species, that were expected to have larger flight muscles, the main role of flight muscles is carrying additional abdominal weight, and not being a source of energy (Helms and Kaspari 2015).

Queens' death associated with entomopathogenic fungi such as *Metarhizium anisopliae* and *Aspergillus flavus* was registered in low frequency, and these fungus species are commonly found in colonies of leaf-cutting ants (Hughes et al. 2009). Colony contamination by these fungi is frequently associated with foraging activity (Currie et al. 1999; Fernández-Marín et al. 2004; Hughes et al. 2009). However, founder queens in this study did not forage outside the chamber suggesting that contamination occurred either during the nuptial flight (Hughes et al. 2009; Marti et al. 2015) or even in the mother colony (see Currie et al. 1999).

To summarize, present data clearly showed a high mortality initially experienced by *Ac. subterraneus* queens, especially for the lightest ones. Although literature repeatedly refers to nesting site competition, excavation, predation and desiccation during foraging as high mortality promoters, the queens' post-flight weight, the non-regurgitation of the symbiotic fungus and, to a lesser extent, the contamination by pathogenic fungi are factors that reduce the success rate of founders and should be equally considered. Suppression of excavation and food search activities seems to have been sufficient to equate offspring production between queens with different post-flight weights, demonstrating that post-flight weight by itself plays an important role in colony foundation success.

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