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ARE THE KEYSTONE INDIVIDUALS THE ACHILLES' HEEL OF THE ANT COLONIES?

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas (Zoologia) - Comportamento e Biologia Animal da Universidade Federal de Juiz de Fora, como parte dos requisitos necessários à obtenção do Título de Mestre.

Orientador: Dra. Juliane Floriano Lopes Santos

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Orientadora: Profa. Dra. Juliane Floriano Lopes Santos

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Comportamento e Biologia Animal da Universidade Federal de Juiz de Fora, como parte dos requisitos necessários à obtenção do Título de Mestre em Ciências Biológicas (área de concentração: Comportamento e Biologia Animal)

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RESUMO ACESSÍVEL

Dentro de um formigueiro, as operárias precisam realizar diferentes tarefas, como cortar as folhas, limpar a colônia, cuidar dos ovos e larvas, e retirar o lixo. Para fazer todas essas atividades com eficiência, elas dividem o trabalho entre si. Porém, essa divisão não é homogênea, isto é, alguns poucos indivíduos trabalham muito enquanto que a maioria trabalha pouco ou até mesmo não trabalha. Isso acontece porque as operárias respondem de maneira diferente aos estímulos do ambiente. Nesse contexto, algumas operárias são consideradas indivíduos-chave por realizaram uma determinada tarefa em um número de vezes maior que outras. Há indícios que estes indivíduos podem influenciar o nível de atividade das companheiras de ninho fazendo com que elas trabalhem mais ou menos. Por outro lado, os indivíduos-chave podem ser o ponto fraco da colônia, ou seja, o Calcanhar de Aquiles da colônia, pois sua ausência ou erro pode colocar em risco o sucesso do formigueiro. Sendo assim, pesquisamos como os indivíduos-chave de uma espécie de formiga cortadeira conhecida como quem-quém (*Acromyrmex subterraneus*) influenciam (i) o grau de atividade das demais operárias e (ii) a eficiência do transporte de folhas (forrageamento) para a colônia. Verificamos também se os indivíduos-chave apresentam um estímulo de resposta persistente a curto prazo para a tarefa de transportar folha. Para isto, marcamos todas as operárias médias de cinco colônias e registramos a frequência de transporte de folhas em cinco ambientes sociais nos quais manipulamos a presença e a ausência dos indivíduos-chave. Os resultados mostram que a eficiência de cada viagem transportando folha é a mesma para os indivíduos chave e as demais operárias, isto é, a quantidade de folha/segundo que cada formiga leva para o formigueiro é a mesma. No entanto, ao remover os indivíduos-chave, operárias que não trabalhavam antes passam a trabalhar e tem-se o aumento da Taxa Total de Entrega de Folhas, pois mais indivíduos fazem a tarefa mesmo que em número menor de vezes. As operárias menos ativas aumentaram o esforço de forrageamento e operárias que antes não transportavam folhas, começaram a trabalhar na ausência dos indivíduos-chave. Com o retorno dos indivíduos-chave, a Taxa Total de Entrega de Folhas das operárias menos ativas, diminuiu, mas ainda continuou maior que a Taxa Total de Entrega de Folhas dos individuos-chave quando comparado com o ambinete inicial, ou seja, antes da remoção dos indivíduos-chave. Sendo assim, quando consideramos a eficiência das categorias no forrageamento, percebemos que há uma maior eficiência para a categoria dos individuos-chave. Porém, estes não podem ser considerados o Calcanhar de Aquiles da colônia, visto que o fornecimento de folhas, isto é, o transporte de folhas para o formigueiro, foi mantido por intermedio da substituição dos individuos-chave por operárias que se tronaram mais ativas e outras que começaram a trabalhar.

RESUMO

Em colônias de formigas, os diferentes limiares de resposta à tarefas levam a uma distribuição não homogênea do trabalho. Algumas operárias, chamadas de indivíduos-chave, executam uma determinada tarefa em um número de vezes maior que as demais. Sendo que estes indivíduos são altamente influentes ao interferir no nível de atividade dos companheiros de ninho. Assim, embora promovam grandes benefícios, os indivíduos-chave podem se tornar pontos de suscetibilidade da colônia e, em seguida, serem considerados como o Calcanhar de Aquiles, cuja ausência ou erro pode colocar em risco o sucesso da colônia. Utilizando o forrageamento como modelo, investigamos como as operárias de *Acromyrmex subterraneus* com alto nível de atividade no transporte de folha influenciam (i) o grau de atividade das demais operárias e (ii) a eficiência do forrageamento da colônia. Além disso, verificamos se o limiar de resposta dos individuos-chave para a tarefa de forrageamento é persistente a curto prazo. Para tanto, marcando individualmente todas as operárias médias de cinco colônias, e registramos a freqüência de transporte de discos foliares em cinco ambientes sociais nos quais manipulamos com a presença e a ausência dos indivíduos-chave. Verificamos que a taxa de entrega foliar (TTF) das três categorias foi semelhante no ambiente social inicial. No entanto, na ausência dos indivíduos-chave, os trabalhadores menos ativos tiveram uma eficiência / viagem de forrageamento semelhante ao ambiente social inicial. No entanto, após o retorno dos individuos-chave, estes apresentaram um menor TTF em comparação com o TTF antes da manipulacçao e com as operárias menos ativas. Na ausência dos indivisuos-chave, as operárias menos ativas aumentaram seu esforço de forrageamento e os indivíduos não forrageadores começaram a trabalhar. Por outro lado, quando considerando a eficiência da categoria no forrageamento, registramos uma maior eficiência para a categoria dos individuos-chave. No entanto, estes não são considerados o Calcanhar de Aquiles da colônia em *A. subterraneus*. Uma vez que o suprimento de folhas foi mantido através de sua substituição por outras operárias, que se tornaram mais eficientes e pelas novas forrageiras ativas.

Palavras-chave: Acromyrmex, Auto organização, Eficiência de forrageamento, Formigas cortadeiras.

ABSTRACT

In ant colonies different task response thresholds lead to a non-homogeneous distribution of the labor within workers. Some workers, called keystone individuals, perform a determinate task in a number of times greater than the others. These individuals are highly influential by interfering with the activity level of nestmates. Thus, although promoting great benefits, keystone individuals can become points of susceptibility of the colony and then be considered as the Achilles' Heel, whose absence or error can put in risk the colony success. Using foraging as a model, we investigated how *Acromyrmex subterraneus* workers with a high level of activity in the leaf transport influence (i) the degree of activity of the other workers and (ii) the colony foraging efficiency. Also, we verified if the task response threshold of keystone workers is short term persistent. By individually marking workers from five colonies, we registered the frequency of leaf discs transport in five social environments in which we manipulated the presence and absence of the keystone individuals. We verified that leaf delivery rate (LDR) of the three categories was similar in initial social environment. However on absence of the keystone individuals, less active workers had similar efficiency/foraging trip to initial social environment. However, after the keystone individuals return, the presented lower LDR in comparison with before the manipulation and than from less active workers. When keystone individuals were absent, less active one increased their foraging effort and non-active individuals started to working. On the other hand when, considering the category efficiency, we registered higher efficiency for keystone categories. However, they were not the Achilles' heel of the colony in *A. subterraneus,* since the leaves supply was maintained through their replacement by other workers, who became more efficient and also by new active foragers.

Keywords: Foraging efficiency, Self organization, *Acromyrmex*, leaf-cutting ants.

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MirmecoLab – Laboratório de Mirmecologia

UFJF – Universidade Federal de Juiz de Fora

SUMMARY

INTRODUCTION

Division of labor is a characteristic Inherent feature in all ant species in which individual workers contribute at different levels for the execution of tasks to the overall success of the colonies (JULIAN & CAHAN 1999; DORNHAUS, 2008; ABRAMOWSKI et al., 2011; PINTER-WOLLMAN et al. 2012; ROCHA et al., 2014; CHARBONNEAU & DORNHAUS, 2015ab; SANTOS et al., 2018). The workers allocation within the tasks occurs without a central control (BONABEAU et al., 1997) by a self-organized system which is based in variations of worker task preference profiles (GORDON, 1996), promoted by different task response thresholds (ROBINSON, 1987; BONABEAU et al., 1996).

In ant colonies, it is observed a reduced number of individuals which tend to be more active, it is also observed a high rate of inactivity, and with about 50 to 70% of the workers remaining inactive for certain tasks (CHEN, 1937; JAISSON et al., 1988; GORDON, 2002; GORDON et al., 2005; DORNHAUS, 2008; DORNHAUS et al., 2008; CHARBONEAU & DORNHAUS, 2015a). For example, during nest digging activity in *Acromyrmex subterraneus* (Forel 1893), such a heterogeneous division of labor is observed, with about 45% of inactive workers (SANTOS et al., 2018).

On the other hand, there are workers who perform high number of task than expected considered as specialists, hyperspecialists or elite (ROBSON & TRANIELLO, 1999; DORNHAUS et al, 2008; PINTER-WOLLMAN et al. 2012; ROCHA et al., 2014; SANTOS et al., 2018).

In *Temnothorax* Mayr, 1861, few keystone individuals perform most of the emigration tasks (DORNHAUS, 2008; DORNHAUS et al, 2008; PINTER-WOLLMAN et al., 2012), being the colony extremely dependent on these (DORNHAUS et al, 2008). These keystone workers exhibit a low response threshold for this task. In *Acromyrmex,* it has been observed that few individuals are responsible for tasks such as the removal of corpses (JULIAN & CAHAN 1999), cleaning of contaminated fungus garden (ABRAMOWSKI et al., 2011) and nest digging (SANTOS et al., 2018).

The variation of response threshold of task among workers allows the emergence of a wide array of behavioral types within a colony (PINTER-WOLLMAN et al., 2012). However, despite of the presence of workers with different response threshold, there will be an uneven distribution of work per individuals within a task (JAISSON et al. 1988; RETANA & CERDA 1991; GORDON et al. 2005; DORNHAUS et al., 2008; BEVERLY et al. 2009; DORNHAUS et al. 2009; SANTOS et al., 2018). Further, it is not possible to predict how frequent or for how long these keystone individuals will remain fulfilling a particular activity (ROBINSON, 1987; BONABEAU et al. 1996).

Keystone individuals (specialists and hyperspecialists) are highly influential in the colony by interfering with the activity level of nestmates (DORNHAUS et al., 2008; MODLMEIER et al., 2014). Thus, although promoting great benefits, keystone individuals can become points of susceptibility of the colony, by diminishing the performance of the group (MODLMEIER et al., 2014) and then be considered as the Achilles' Heel, whose absence or error can put in risk the success of the colony (PRUITT et al., 2016).

Using foraging as a model and based on the non-homogeneous division of labor among *A. subterraneus* workers (SANTOS et al., 2018), we investigated how workers with a high level of activity in the leaf transport task influence (i) the degree of activity of the other workers and (ii) the colony foraging efficiency. Also, we verified if the task response threshold of keystone workers is short term persistent.

MATERIAL AND METHODS

Studied Colonies

Five *A. subterraneus* colonies maintained since October 2017 in the Laboratory of Myrmecology, Federal University of Juiz de Fora (UFJF), Juiz de Fora, Minas Gerais, Brazil, were used. They are maintained in a closed system consisting of three compartments. There are: the fungus chamber, the foraging arena, and the waste chamber, which are interconnected by transparent plastic tubing. Laboratory conditions were kept at a 26 ºC temperature, 70 % relative humidity and *Acalypha wilkesiana* (Euphorbiaceae) leaves were offered daily, as symbiotic fungus substrate. Furthermore, the colonies were not fed for 24 h before each experimental event to ensure high foraging activity (LOPES et al., 2004; HERZ et al., 2008) (Fig. 1).

Figure 1 - (A) Colonies of *Acromyrmex subterraneus* mantnied in MirmecoLab of UFJF; (B) closed system consisting of three compartments. There are: the fungus chamber, the foraging arena, and the waste chamber, which are interconnected by transparent plastic tubing and (C) leafs of *Acalipha wikesi*ana used for alimentation of the colonies.

Experimental procedure

Medium size workers, with head width ranging from 1.2 to 1.6 mm (HASTENREITER et al., 2015; SANTOS et al., 2017), were selected. To identify each worker, they were marked with a unique color combination on the pronotum and abdomen using Edding® markers, due to their excellent adhesion, rapid drying and good visibility (CAMARGO et al., 2007) (Fig. 2). A total of 51 workers were marked at Colony 1, 46 at Colony 2, 51 at Colony 3, 47 at Colony 4 and 61 at Colony 5, totaling 256 individuals.

Figure 2 - *Acromyrmex subterraneus* workers marked with a unique color combination on the pronotum and abdomen using Edding® markers for identification individual.

The foraging arena of each colony was replaced by a new rectangular one, connected by a 50 cm long glass bridge to the colony. A transversal line, 5 cm from the exit of the colony, was established to register the instant of exit (Texit) and return (Treturn) for each marked worker with a chronometer, allowing to calculate their foraging trip time (Fig. 3). At the foraging arena, we supplied 100 discs of *A. wilkesiana* (0.5 cm diameter) cut with a manual metal punch from fresh leaves harvested prior to the experiment (Fig. 4). Then when all workers were allowed to pass through the arena, the time was recorded and we registered the frequency of leaf disc transport for each marked worker, until all the leaf discs had been transported, or up until who completed two hours of foraging.

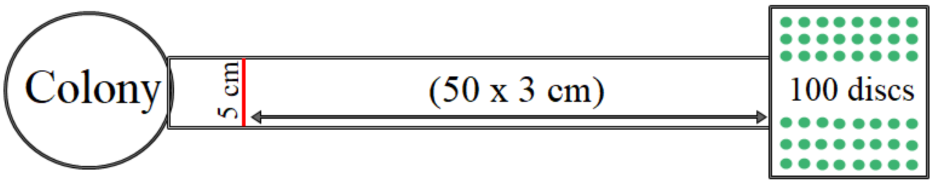

Figure 3 – Experimental set-up, showing the colony connected to the foraging arena by a glass bridge.

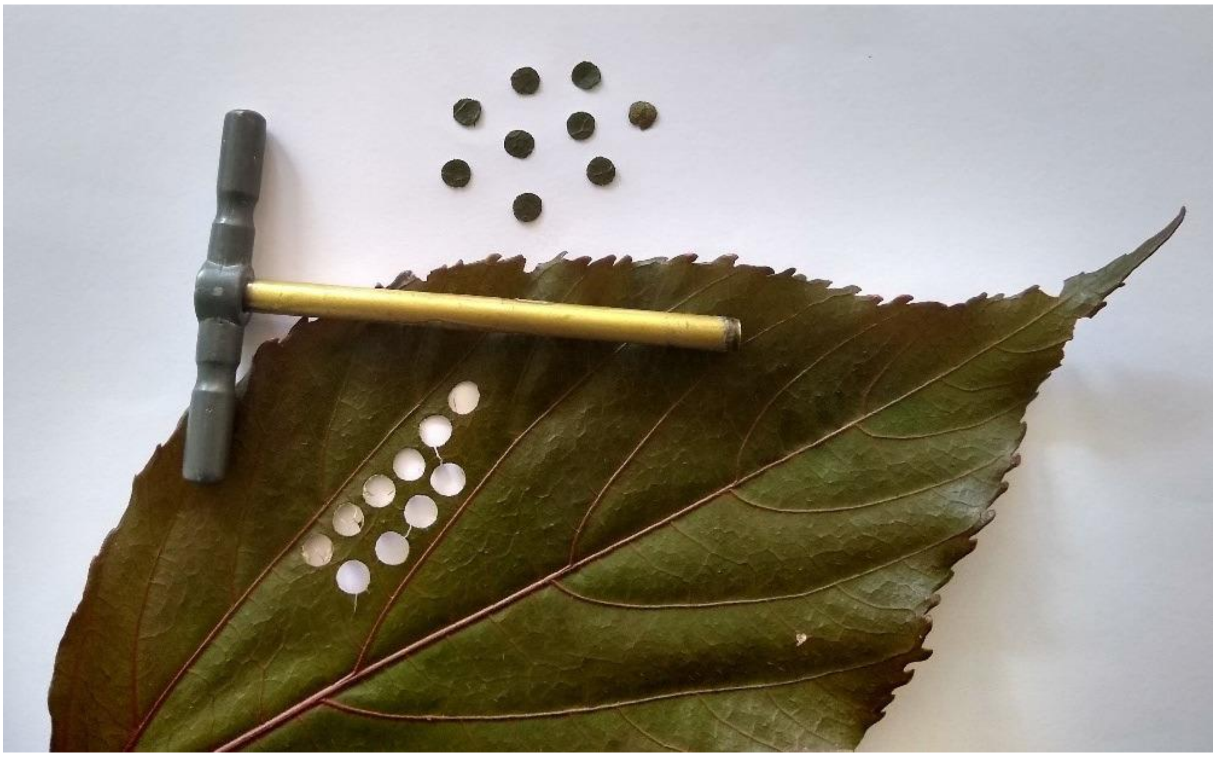

Figure 4 – Cut of *Acaplypha wilkesiana* fresh leaf with a manual metal punch to provide the leaf discs prior to the experiment.

To categorize workers according to their foraging activity level, we first calculated a specialization baseline arbitrarily corresponding to 1.5 times the mean number of leaf disc transports per worker (Equation1) (see CORBARA et al. 1989, SCHATZ et al. 1995 and SANTOS et al., 2018 for a similar treatment). Similarly, we also calculated a hyperspecialist baseline value which now corresponded to more than 1.5 times the mean number of leaf disc transports per worker that performed this task (only those ones who foraged) (Equation 2) (Santos et al., 2018, Rocha et al. 2014). Thus workers that transported less leaf discs than the specialist baseline value were considered generalists, and those that did not transport, as non-forager.

Equation 1:

$$\text{Specialists} = \frac{\text{Total of transporting of discs leaf}}{\text{Number of workers}} \times 1{,}5$$

Equation 2:

$$\text{Hyperspecialists} = \frac{\text{Total of transporting of discs leaf}}{\text{Number of workers who transported the disks}} \times 1{,}5$$

At this point, we had data about the worker foraging activity level at what we designated as the control social environment and so we initiated the manipulation of different social environments, which differed in presence and absence of keystone individuals (specialists and hyperspecialists). Each social environment was observed on average for 15.26 hours for five consecutive days at the five colonies, totalizing 76.29 hours of observation. So, the isolation period for removed worker categories was two weeks.

- ✓ **Control Environment:** presence of hyperspecialist (H), specialist (S), generalist (G) and non-forager (nF). Used to categorize workers according to their foraging activity level;
- ✓ **SGnF Environment:** Removal of H (presence of categories S, G and nF);
- ✓ **GnF Environment:** Removal of S (presence of categories G and nF);
- ✓ **HGnF Environment:** Returnal of H (presence of categories H, G and nF);
- ✓ **Restored Environment:** Returnal of S (restoration of initial social environment).

Removal and return of keystone individuals were done by picking the respective marked worker with a narrow tip featherweight forceps (Bioquip #4748). These were held on after the last repetition of each social environment. After the manipulation, the colonies remained intact during 48 hours before the simulation of next social environment in order to avoid the stress of the workers. While out of the colony, workers were kept in pots of 200 cm³ containing 1cm of plaster to maintain humidity, a portion of symbiotic fungus was also supplied with leaves daily (Fig. 5).

Figure 5 - Minicolonies (pots) used for maintenance of *Acromyrmex subterraneus* workers hyperspecialists (H) and specialists (S) during the isolation period.

A standard burden was determined by weighting 50 medium size forager workers and 50 leaf discs after dehydration in an oven at 70ºC for 24 hours (Fig. 6), and applying the Burden formula (Equation 3) (DUSSUTOUR et al., 2007; SALES et al., 2015).

Equation 3:

$$\text{Burden} = \frac{(\text{Leaf dry weight} + \text{worker dry weight})}{\text{Worker dry weight}}$$

Figure 6 - (A) Oven used for the dehydration of workers and leaf discs and (B) analytical balance used for weighing leaf discs and *Acromyrmex subterraneus* workers.

The travel time (TT) corresponds to the difference between Treturn and Texit of each marked worker transporting a leaf disc (Equation 4). To calculate the efficiency of each worker category activity, we used the Efficiency formula adapted from DORNHAUS (2008) (Equation

5). To calculate the Leaf Delivery Rate (LDR), which is considered a foraging performance measure (BURD, 2000), we used the formula LDR adapted from RUDOLPH and LOUDON (1986) (Equation 6).

Equation 4:

$$\mathrm{TT} = \mathrm{Treturn} - \mathrm{Texit}$$

Equation 5:

$$\mathrm{Efficiency} = \frac{(\text{Number of trips by category x Burden})}{\text{TT mean}}$$

Equation 6:

$$\mathrm{LDR} = \frac{Burden}{\mathrm{TT}}$$

We highlight that LDR provides a measure of plant material input to the colony for each trip made by workers from each category at each social environment and do not consider how many individuals belong to the activity categories or how many foraging trips they made.

Statistical analyses

We used a generalized linear mixed model (GLMM) to compare response variable among treatments. Colony was entered as a random factor and a Tukey post hoc test was used for pairwise comparisons between treatments. To investigate (i) the differences among worker categories foraging effort and between Control and Restored Environments the LDR was compared with a GLMM, using a Gamma distribution. The same was applied to verify (ii) differences in LDR of generalist workers, (iii) in the efficiency among activity worker categories and social environments and (iv) in the relative frequency of non-forager among all social environments. The proportion in which (v) workers shifted between categories was compared by a $\chi 2$ test for independence. Also, we calculated the association coefficient that allow us to verify the degree of association among the variables (BARBETTA, 2001). All statistical tests were run with the software R 3.5.1 (R Core team, 2018) and the packages chorddiag (FLOR, NA), devtools (WICKHAM et al., 2018), dplyr (WICKHAM et al., 2018), ggplot2 (WICKHAM, 2016), ggpubr (KASSAMBARA, 2018), jtools (LONG, 2018), lattice (DEEPAYAN, 2008), lme4 (BATES et al, 2015), multcomp (HOTHORN et al., 2018), muMin (BARTON, 2018), nlme (PINHEIRO et al., 2018), RColorBrewer (NEUWIRTH, 2014) and scales (WICKHAM, 2018).

RESULTS

Foraging activity of individually marked workers showed that in mean 48.73% of the observed individuals were engaged on this task (Tab. 1). The proportion of Non-forager workers varied between 33.30 to 60.66%, corresponding to an average of 46.76% individuals. The colonies were composed on average of 27.14% of keystone individuals (Tab. 1).

Table 1 - Mean percentage of *Acromyrmex subterraneus* workers in each category according to their foraging activity.

Colony	Non-forager	Generalists	Specialists	Hyperspecialists
C1	33.30	35.29	11.76	19.61
C2	45.65	15.22	28.26	10.87
C3	43.14	23.53	11.76	21.57
C4	51.06	19.15	12.77	17.02
C5	60.66	14.75	13.11	11.48
Mean	46.76	21.59	15.53	11.61

The LDR was significantly lower at Restored Environment when compared with the Control Environment (AIC = -25547.50; t = -124.09; $p < 0.001$), meaning that even after restoring the social environment by returning the S and H workers they did not work at the same effort level as in the control. In fact we verified a significant interaction between worker category and social environment, showing that the social environment manipulation interferes with the variation of the LDR in relation to the worker category. We observed that after the keystone individuals returnal, these presented a lower LDR than before the manipulation and then from G workers (Fig. 7).

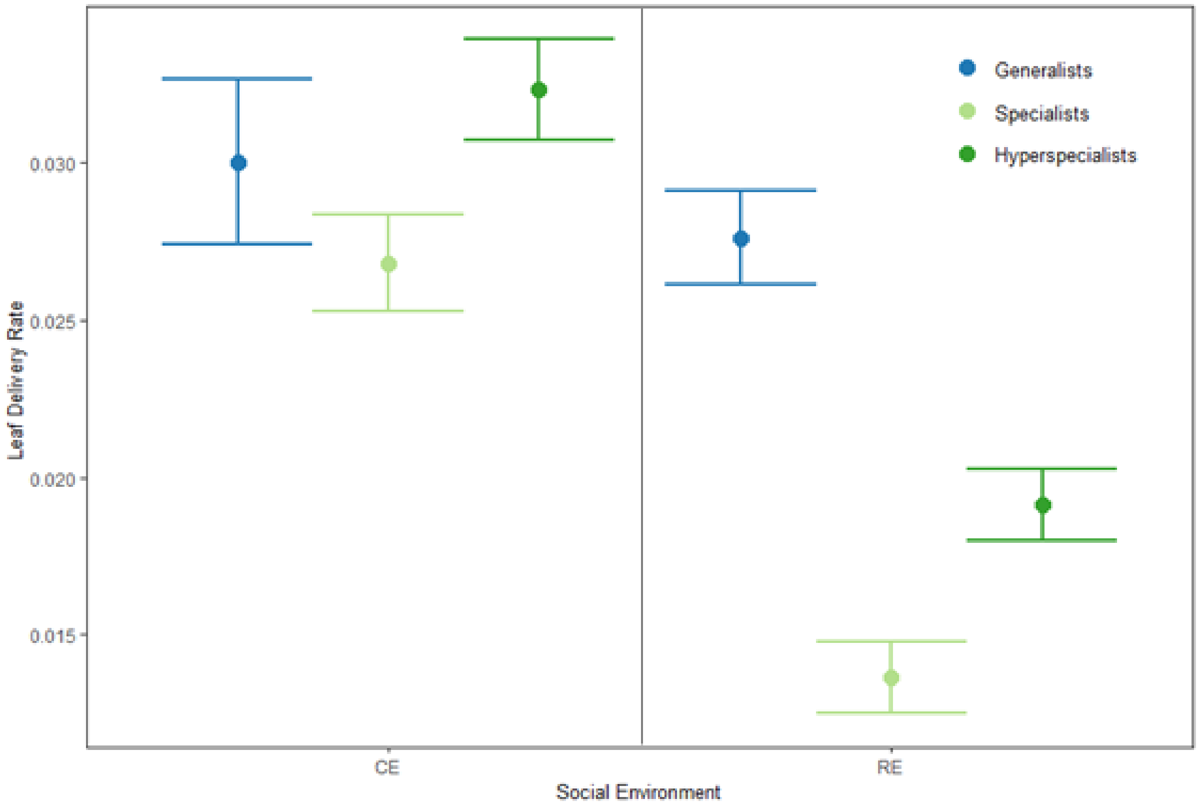

Figure 7 - Mean Leaf Delivery Rate (LDR) of *Acromyrmex subterraneus* worker categories Generalists (G), Specialists (S) and Hyperspecialists (H) in two social environments: Control Environment (CE) – before keystone individuals removal and Restored Environment (RE) - after keystone individuals return (RE).

Analyzing just generalist workers, there was a significant difference for LDR among the different social environments (AIC = -29647.80; t = -69.59; p <0.001). LDR was significantly higher at the GnF Environment and significantly lower at Restored Environment in relation to the other social environments. As we used a standardized burden, a higher LDR indicates that generalist workers reduce their travel time in the absence of the keystone individuals. On the other hand, at the restored environment the time spent we registered a lower LDR, indicating that time spent outside the nest is even greater than before (Fig. 8).

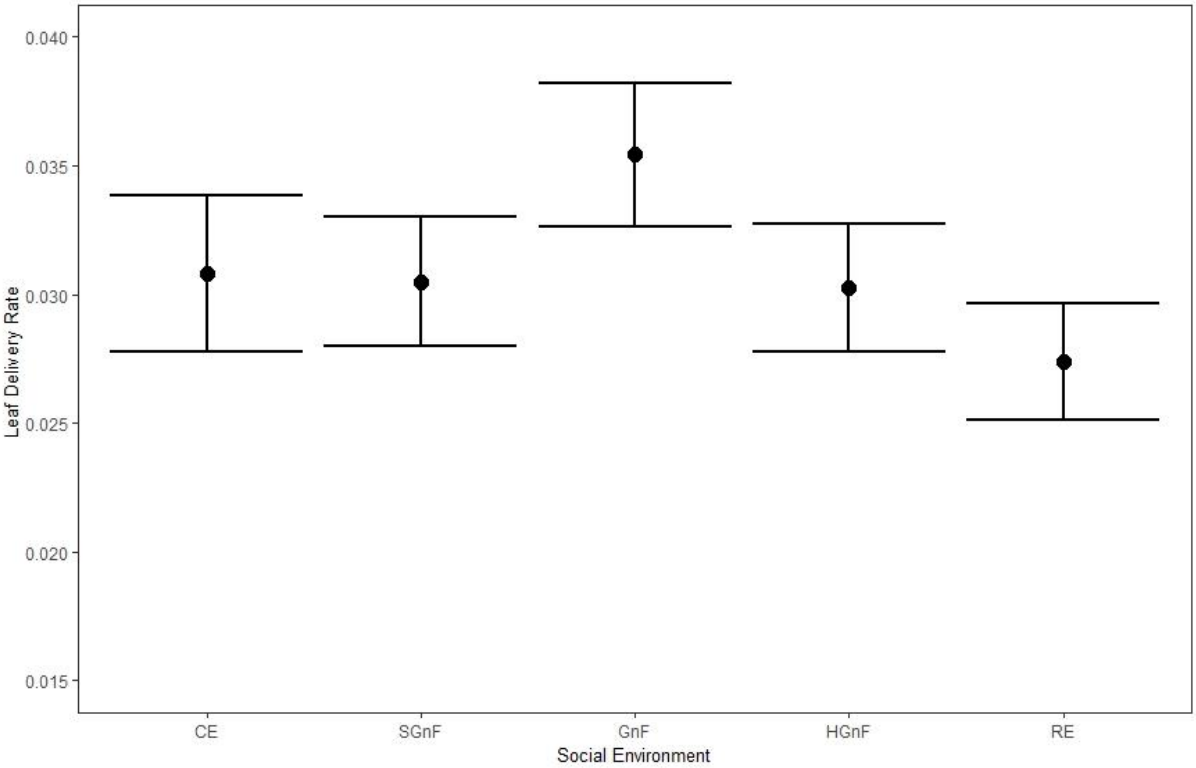

Figure 8 - Mean Leaf Delivery Rate of *Acromyrmex subterraneus* generalist workers in the manipulated social environments: Control Environment (CE); SGnF Environment; GnF Environment; HGnF Environment; Restored Environment (RE).

In the Control Environment, H workers were more efficient than S and G. In the SGnF Environment, where H individuals were absent we registered a higher efficiency of S and G and that some nF workers start to forage (now called New Active (NA)). In the GnF Environment, where all keystone individuals (H and S) were absent, the efficiency of G and NA workers was even higher than in any other social environment. With the return of H workers in the HGnF Environment, efficiency decreased for all workers (G, H and NA). However G workers presented higher efficiency than the others. Even with the presence of all keystone individuals (S and H) in the Restored Environment, the efficiency of G workers remained higher than the others (S, H and NA). Summarizing, the G workers increased the number of trips and NA individuals were seen foraging in the absence of the keystone individuals (Fig. 9).

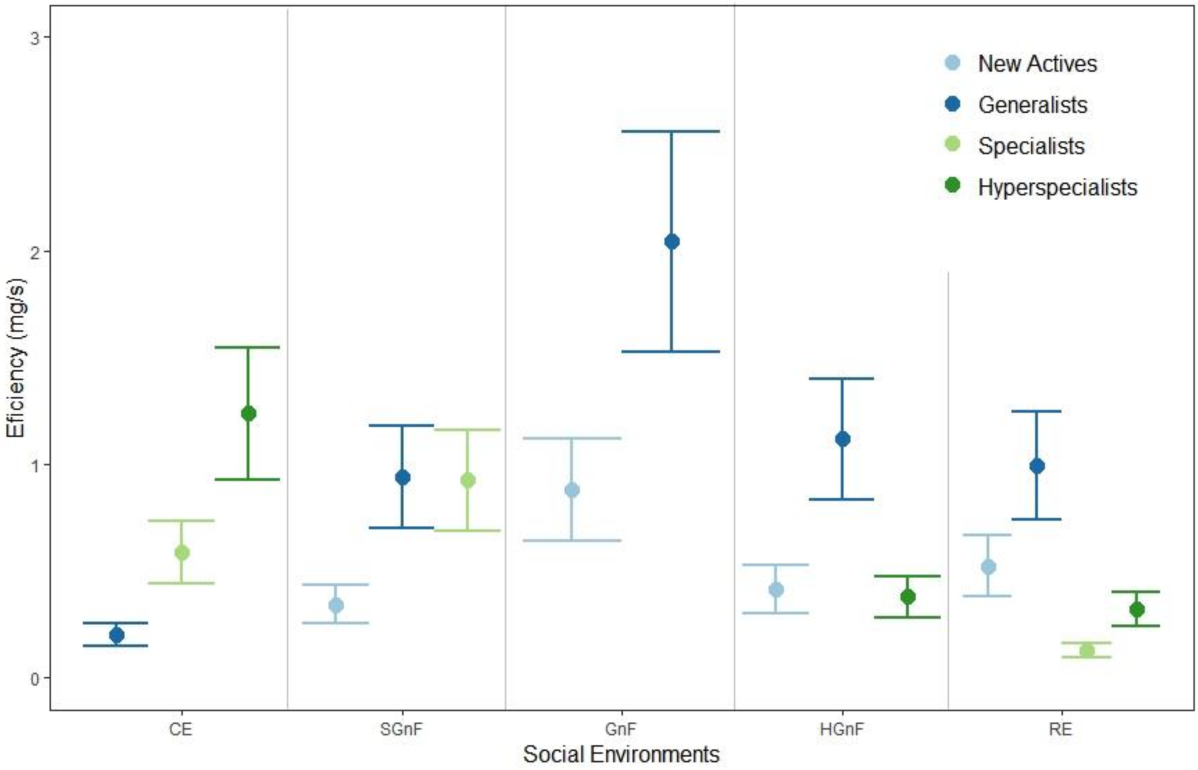

Figure 9 - Mean foraging efficiency (mg/s) of *Acromyrmex subterraneus* worker categories: Generalists (G), Specialists (S), Hyperspecialists (H) and New Actives (NA) in Control Environment (CE), EGnF Environment, GnF Environment, HGnF Environment and Restored Environment (RE).

Also, at the GI Environment, had was a significant decrease of the proportion of nF workers (AIC = 893.96; t = 36.99; $p < 0.001$), what indicates that the absence the keystone individuals induces them to work. Similarly, when the social environment was restored there was an increase in the proportion of nF workers (Fig. 10).

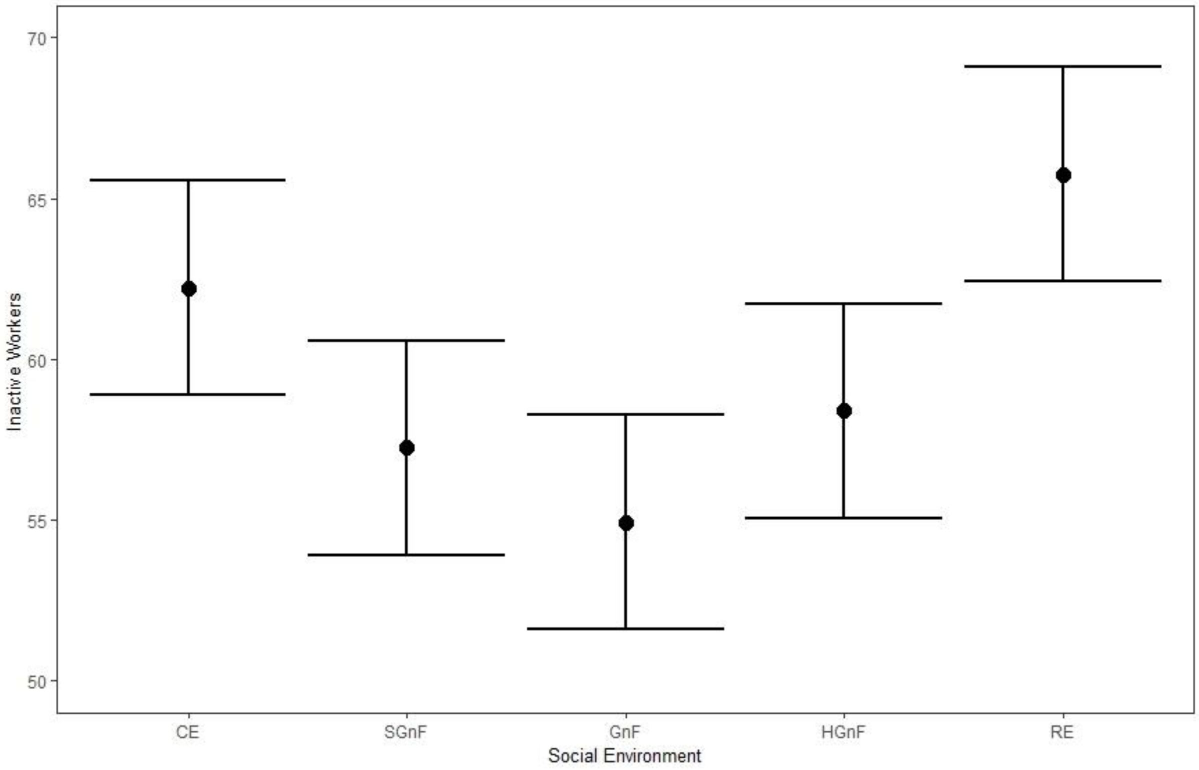

Figure 10 - Mean proportion of non-foragers workers of *Acromyrmex subterraneus* in Control Environment (CE), EGnF Environment, GnF Environment, HGnF Environment and Restored Environment (RE).

The percentage of workers that shifted between categories was not independent ($\chi2$ = 85.55; $p < 0.001$), indicating that there is a relationship between the categories. According to the contingency coefficient (0.96), the association was moderate for category shifting. In fact, we observed through the proportions of category shifting that E had more tendency to become G and nF and G to become nF (S – G: 30.90%; S – nF: 44.44%; G – nF: 58.18%), meaning that they reduced their activity. On the other hand, H workers presented even or equal shift tendency to any category (H – nF: 27.5%; H – G: 27.5%; H – S: 25% e H – H: 20%). Non-foragers workers tended to remain nF (85%) (Fig. 11).

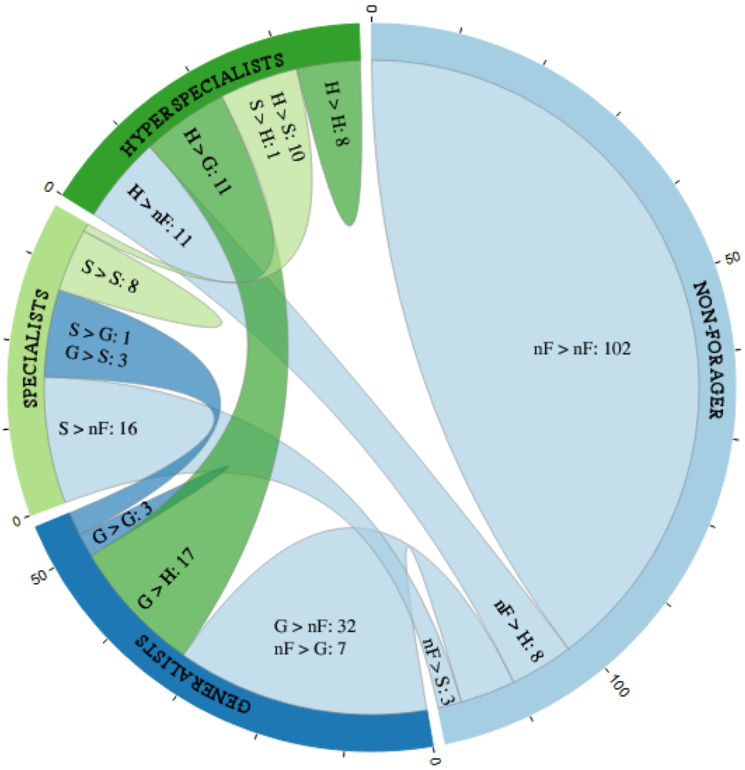

Figure 11 - Activity category shifting of *Acromyrmex subterraneus* workers after restoration of the social environment. Numbers outside the circle diagram indicates the number of individuals of each category before shifting. Band width is proportional to the number of workers which shifted from one category to another

DISCUSSION

The removal of the keystone individuals (S and H) promoted an increase in the activity of the workers that before the removal had lower activity level (G) and even did not participate in the foraging activity (nF), according to the response threshold model proposed by Theraulaz et al. (1998). However, there was a reduction in foraging efficiency, since the keystone individuals, after being reintroduced, presented a lower foraging performance, indicating that their response threshold was not persistent and changed over time, due to the manipulation of social environments.

The LDR of the three categories of workers was similar in the Control Environment, showing that the contribution of each foraging trip was the same independent of a worker belonging to one or another activity category. Since the burden was standardized by offering discs and evaluating workers of the same size, we can conclude that the time spent outside the nest was similar, and so was the performance/trip. In this context, we must reject the general assumption presented in many previous studies with social insects that specialists are more efficient than generalists (PORTER & TSCHINKEL, 1985; CALABI & TRANIELLO, 1989;

O' DONNELL & JEANNE, 1990; TRUMBO & ROBINSON, 1997; JULIAN & CAHAN, 1999; DORNHAUS, 2008). In fact, in the same way as registered in the present study, *Temnothorax albipennis* specialist workers are not more efficient than generalists during colony emigration (DORNHAUS, 2008).

On the other hand, the LDR of the keystone individuals (S and H) decreased in the Restored Environment while remained unaltered for generalists (G), indicating that keystone individuals spent more time foraging. This decay suggests that these individuals became less predisposed to transport the leaf discs and so we can say that their response threshold was not persistent and so the same stimulus was no longer sufficient to trigger their previous activity level. It would be reasonable to wonder that they became slower because their nestmates are already allocated to the task. However, generalists maintained the LDR and the number of nF workers decreased when the keystone individuals were restored to the colony.

Thus we must analyze this result under the foraging for work theory (FFW: TOFTS & FRANKS, 1992). According with this theory, workers search for unexecuted tasks, and this search leads to the processing of these tasks. In this way, workers must access colony needs, what is done through direct interactions with their nestmates between periods of task performance, when they collect and integrate the information on the status of a subset of activities (ROBINSON, 1992), as if they are sampling behavior inside the nest. In fact, this sampling is facilitated because ant colonies present synchronous bursts of worker activity (HÖLDOBLER & WILSON, 1990).

But how workers would access information about colony needs if they were kept isolated for two weeks? Without current information about colony needs, they did not reach the same activity level. In fact the reinforcement - continuous repetition – decreases the response thresholds over time (WEIDENMULLER 2004). In this study, keystone individuals were deprived from the foraging stimulus while they were isolated, suggesting that the negative reinforcement could act increasing their response threshold.

The same concept could be used to explain generalist LDR variation along the different social environments. Generalists maintained their effort in the restored environment due to the positive reinforcement, and exhibit an enhanced effort when keystone workers were absent.

Keystone individuals may be of extreme importance to the colony, as noted in *T. albipennis* for the task of emigration (DORNHAUS et al, 2008) and in social spider *Stegodyphus dumicola* Pocock, 1898 (PRUITT et al., 2016). However, the contribution of them in *A. subterraneus* colonies considering each foraging trip seems not to be weakness and they are not the Achilles' heel of the colony.

The removal of the keystone individuals promoted an increase in the travel time of generalist workers (GnF Environment), which allowed a higher LDR in comparison to that calculated in the other social environments. This improvement at foraging effort of G workers could results in individuals highly prepared for the task (LANGRIDGE et al. 2008; PINTER-WOLLMAN et al. 2012; PRUITT et al., 2014; ROCHA et al., 2014; LARSEN et al., 2016) what is confirmed by the maintenance of the LDR after keystone individuals returned.

There was also an increase in the proportion of nF workers who began to forage after the keystone individuals removal, possibly due to leaf entry reduction. The reduction of leaves input could act as a stimulus sufficiently big enough to surpass their high response threshold. Individuals could be non-foragers because their response threshold is high for such task and probably low for intra-nest tasks, such as communication center, trophic egg production, brood care (CHARBONNEAU & DORNHAUS, 2015ab; CHARBONNEAU et al., 2017b), fungus cultivation or colony cleaning (FORTI et al., 2004). Which are the necessary tasks for the colony function in short time scales (CHARBONNEAU et al., 2017a), or even maintenance of physical trail (JAFFÉ & HOWSE, 1979).

The finding that 46.77% of the workers were inactive for foraging is in accordance with Santos et al. (2018) and Gordon (2002). In particular, these workers serves as an extra worker contingent ready to replace and restore the worker force in the case of loss of active workers (PINTER-WOLLMAN et al. 2012; LARSEN et al., 2016; CHARBONNEAU et al., 2017ab). So they can start to work immediately after the removal of keystone individuals. As in *T. albipennis* (PINTER-WOLLMAN et al. 2012), *Myrmica kotokui* Collingwood, 1976 (ISHII & HASGEAWA, 2013; HASEGAWA et al., 2016) and *Temnothorax rugatulus* (Emery, 1895) (CHARBONNEAU et al., 2017a), previously inactive *Acromyrmex* workers began working immediately when active workers are removed.

Replacement of keystone individuals by new active ones provides an effective mean for the continuous processing of tasks, which results at the long-term persistence of a colony (PINTER-WOLLMAN et al. 2012; HASEGAWA et al., 2016) after anthropic (i.e. formicide application) or natural (i.e. burnt) impact. For example, for *Atta bisphaerica* (Forel, 1908) is registered that the foraging activity only started 15 days after the burning of sugar cane dry straw (ARAUJO et al., 2004). Authors suggest that this interval is due to forager workers contingent death. Also is often the resurgence of foraging activity after chemical control when toxic baits are applied in a subdose (JLopes, personal communication).

Non-foragers workers shifted among activity categories probably due to the perception of colony needs, however their returned to foraging inactivity after restoration of the social environment was observed. The execution of a task results in learning for the individuals (CHARBONNEAU & DORNHAUS, 2015a), and they can choose which tasks to perform based on their own experiences, depending on how frequently a worker has performed or successfully performed each task in the past (RAVARY et al. 2007; WEIDENMÜLLER et al. 2009). In this way, workers engaged in intranests activities can return to execute these essential tasks for the maintenance of the colony after demography restoration. Such flexibility may represent an adaptive advantage under natural conditions (ROCHA et al., 2014).

Although hyperspecialists and specialists presented a greater foraging efficiency, our results show that keystone individuals are not the Achilles' heel of the colony in *A. subterraneus*, since the supply of leaves was maintained through their replacement by generalist workers, who became more efficient and also by new active foragers (nF foragers before).

Ant colonies are examples of sophisticated and optimized self-organized systems shaped along the evolution process (CHARBONNEAU & DORNHAUS, 2015a). Observed and modeled strategies can provide a rich resource to inspire better strategies in the allocation of tasks for engineering systems (FEINERMAN & KORMAN 2013; NAVLAKHA & BAR-JOSEPH 2014; ZAMBONELLI et al. 2015) and management of human organizations (PARUNAK, 1997).

CONCLUSION

In colonies of *Acromyrmex subterraneus*, notable what the individuals generalists and new forages in the transport de discs of leaf, which maintenied the supply of leaf. These demonstrated larger efficiency of the four categories here studied in absence of the keystones individuals. Thus, the keystones individuals cannot be considered the Achilles ‘heel of the colonies.

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