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Age Polyethism in *Atta sexdens* (Linnaeus) (Hymenoptera: Formicidae)

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Abstract

This study aimed to verify age polyethism occurrence in medium-sized (cephalic capsule = 2.3 ± 0.21 mm) and small-sized (cc = 1.4 ± 0.10 mm) workers from *Atta sexdens* (Linnaeus) colonies. Four laboratory colonies were used, and they were maintained at 25 ± 2 °C, with 75 ± 3% relative humidity and a 12-hour photoperiod. Workers from these colonies were marked after their emergence and observed throughout their lifetime to determine which tasks they performed. The number of ants performing each activity was analyzed using linear mixed-effect models (LME), considering the temporal effect and the block design (colonies). We found that fungal garden maintenance tasks were frequent for both sizes, but their occurrence decreased significantly from the ninth week. The foraging activity occurred gradually in both sizes, with stabilization in the number of workers from the fourth week onwards and declined in the last three weeks of lifespan. Waste management tasks occurred throughout life but were more frequent during the first two weeks of life, in both medium and small workers. Therefore, age polyethism may be related to all activities; however, foraging tasks presented a distinct pattern compared to tasks in the fungus garden and refuse dump, where younger ants were more frequently observed.

Introduction

Eusociality is characterized by individuals of several generations living together and a non-reproductive caste caring for the offspring, thereby creating a labor division. Social insect colonies are structured in a labor division system based on morphological and behavioral castes (Wilson, 1971; Wilson, 1980).

The high density of related individuals in social insect colonies is expected to make them more susceptible to infections by increasing pathogen transmission rates (Schmid-Hempel, 1998). However, proper social insect organization may reduce the probability of pathogen transmission, thereby reducing pathogen establishment in the colonies (Cremer et al., 2007). If workers can infect queens, e.g., workers can infect queens during their care behavior, their access to them should be restricted since, and mainly when the colony depends on a single queen (monogyny) (Wang & Mofller, 1970). Another pattern observed in several social insect species is labor division based on age. In this case, younger individuals assist the queen while older individuals forage (Wilson, 1971). This labor division is called age polyethism and corresponds to task switching according to individual age. Age polyethism is relatively common in social insects (Kolmes, 1986), including bees (Santos et al., 2010), termites (Yanagihara et al., 2018), and ants (Santana Vieira et al., 2010). Generally, younger individuals perform safer tasks inside the nest, while older workers perform riskier tasks outside, as the latter are "less valuable" to the colony (Santana Vieira et al., 2010).

Age polyethism in ants can be quite distinctive, as well as stereotypical or not. In *Pheidole hortensis* (Forel) colonies, older workers continue to perform characteristics of

young workers (Calabi et al., 1983). It has been shown that, in *Ectatomma opaciventre* (Roger) nests, outside activities such as defense and foraging are held by older workers, while offspring care is performed by the younger individuals (Miguel & Del-Claro, 2005). Age polyethism also occurs in *Dinoponera lucida* (Emery) workers (Peixoto et al., 2008). In the leaf-cutting ants, some studies show that age polyethism is present in *Acromyrmex subterraneus brunneus* (Forel) colonies in which young workers remain inside the nest while older workers perform activities outside the colony (Camargo et al., 2007). Workers of *Atta cephalotes* shift their behavior from leaf-cutting to leaf carrying when they become less effective at cutting leaves as they age and their mandibles become worn (Schofield et al., 2011).

In leaf-cutting ant colonies, workers perform various tasks, such as foraging and offspring, queen and fungus garden care, in addition to defending the colony. There are also hygiene-related tasks, which involve waste transport out of the fungus garden chamber and waste rearrangement in garbage piles. These tasks avoid the spread of waste that could promote disease propagation (Lacerda et al., 2006).

This study aimed to investigate age polyethism occurrence in nests of *Atta sexdens,* commonly known as leafcutter ant. This species is widely distributed in all Brazil regions and is economically significant because it damages agriculture and planted forests.

Material and Methods

We used four *A. sexdens* colonies maintained in the Insectarium of the Federal University of Viçosa at 25 ± 2 °C, 75 ± 3% relative humidity, and a 12-hour photoperiod. The estimated population of each colony was 16,040; 17,620; 20,020 and 14,020 individuals, and colonies were designated as C1, C2, C3, and C4, respectively. Population estimation was based on ant workers count in 50 mL of the fungus garden, and the result was extrapolated to the total volume of fungus garden in each nest.

According to the available offspring, the highest possible number of medium (cephalic capsule width = 2.3 ± 0.21 mm) and small (cephalic capsule width = 1.4 ± 0.10 mm) pupae were withdrawn from each colony. Thus, 308 medium and 250 small pupae were taken from C1 colony; 352 medium and 200 small pupae from C2 colony; 401 medium and 200 small pupae from C3 colony; and 138 medium and 80 small pupae from C4 colony.

Pupae were placed inside mini-colonies in the absence of a queen; each colony had its mini-colony, which consisted of a 200 ml plastic container with one-centimeter gypsum basal layer to maintain the humidity inside. Seventy foraging workers and 200 gardening workers were placed in each mini-colony to provide offspring care. Each container had an opening that allowed worker foraging and waste output and a perforated lid on top of which a cotton swab moistened with distilled water was placed. A 100 mL fungal garden fragment from each colony was placed in each container. This set was placed within a 4 L container whose borders were treated with a Fluon® layer to prevent workers' escape. *Acalypha wilkesiana* Müll. Arg. and *Ligustrum japonicum* Thunb leaves were also placed within the larger containers to serve as the substrate for symbiotic fungi growth. After their emergence in the mini-colonies, adults were adequately marked and put back in their original colony. Because adults did not emerge simultaneously, we marked them with different colors according to their emergence time to facilitate age identification.

The activities of the newly emerged adults were observed 24 hours after they were returned to their original colonies. The fungus garden was placed in rectangular pots with a 192 cm^2 area and 4 cm height to facilitate the marked colony workers' observation. A one-centimeter gypsum layer was also placed at the base of each pot. For each colony, as many pots were used as necessary, according to fungus garden volume and its subsequent growth. Fungus garden volume was approximately 1.5 L for C1 and C4 colonies and 2.0 L for C2 and C3 colonies.

Observation of workers' behavior was performed daily for half an hour in each colony until no marked worker was present in each nest, i.e., when all were already dead or when marks had disappeared. The observed behaviors were fungus garden, offspring, queen care, foraging, and waste management. The first three behaviors were grouped into "fungus garden care" as offspring, and queen care activities were rarely seen.

The number of daily occurrences of each behavior was grouped in weeks for each colony. Because workers emerged at different times, resulting in three age classes for each ant size, the mean of the three ages about behavior occurrence each week and each colony was calculated. Also, for improved interpretability of results, a mean of the four colonies relative to each behavior occurrence in each week was calculated. Age polyethism observation methodology followed the example of Camargo et al. (2007), with modifications, because these authors used only two colonies maintained in a closed foraging system and observed small, medium, and large workers. Linear mixed effect models (LME) were used to compare differences in the number of ants marked in relation to their lifetime (weeks) and the cephalic capsule size. In these models, the number of ants (response variables) were tested with worker size, time (weeks), and the interaction worker size × time. Complete models were fitted with all response variables as fixed effects, whereas colonies were used as random effects (Crawley, 2012).

Results

The behavior of fungus garden care in each week of the experiment was similar between small and medium workers ($F_{1, 87} = 0.34$; $p > 0.05$). The average number of workers performing the task remained stable until the eighth week and decreased from then until the end of the observations,

showing two distinct periods in the activity ($F_{12,87} = 48.97$; $p < 0.001$; Fig 1). There was no significant difference between the two size classes ($F_{1,87} = 0.34$; $p = 0.55$).

The average number of medium and small-sized workers performing foraging activities gradually increased until the fourth week, remaining stable until the 11th week and declining after that until the end of the experiment ($F_{13,94} = 5.35$; $p = 0.02$; Fig 2). There was significant difference between the two size classes, with the medium workers being slightly more frequent than the small ones ($F_{1,94} = 4.54$; $p = 0.03$) whereas the size x time interaction was not significant ($F_{1,81} = 0.25$; $p = 0.9$).

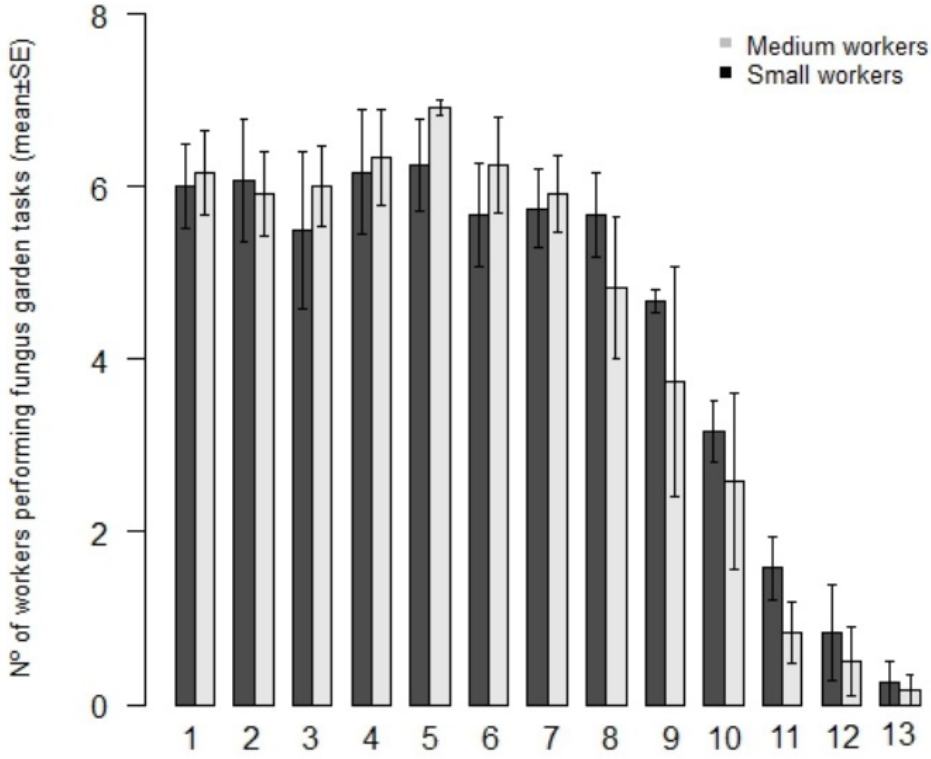

Fig 1. Number of tasks occurrence within the fungus garden (garden, offspring and queen care) each week by medium and small-sized workers (mean ± SE).

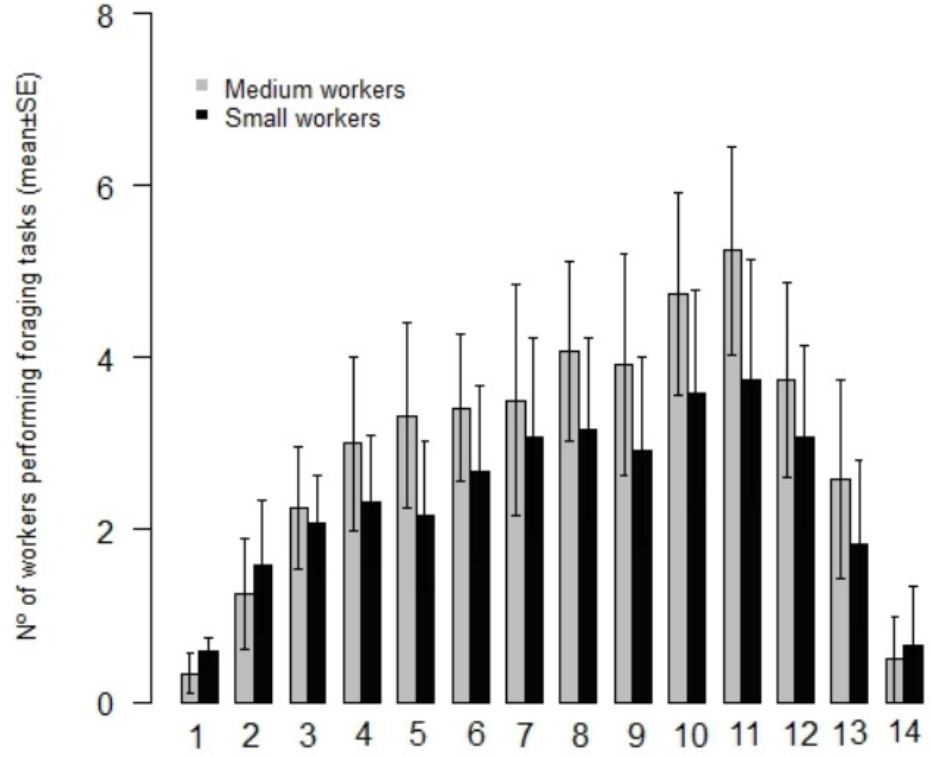

Fig 2. Average number of medium and small-sized workers (± SE) performing foraging tasks each week.

The occurrence of waste management activities performed by workers was more frequent in the first two weeks of post-emergence ($F_{12,87}$ = 3.68; p < 0.001; Fig 3) and was constant until the last two weeks of the experiment, where the lowest values were observed. In general, waste management activities were performed by workers of both sizes ($F_{1,87}$ = 2.04; p > 0.05).

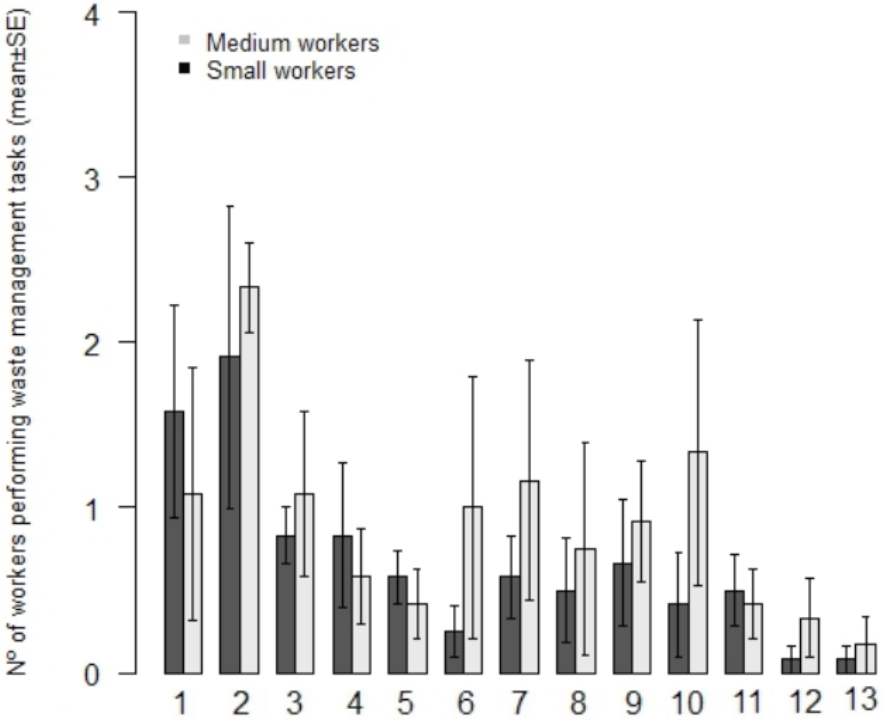

Fig 3. Number of medium and small-sized workers (mean ± SE) performing waste management each week.

Discussion

In *A. sexdens* colonies, we observed that medium and small workers performed both the inside and outside tasks throughout their lives in varying degrees. Inside work, such as fungus garden, offspring and queen care, was typical throughout their lives, although its occurrence decreased around the ninth and tenth weeks in medium and small workers, respectively. There may be a task specialization by those workers who remained caring for the garden until the end of their lives. Because the queen mates multiple times, there is a certain degree of genetic diversity among the workers, and this factor could also partly explain the results obtained here. Genetic-influenced behavioral specialization was found in *Acromyrmex versicolor* (Pergande) (Julian & Fewell, 2004) and *Acromyrmex echinatior* (Waddington et al., 2010). Foraging activities also occurred throughout the lifetime of medium and small workers, becoming more common in the eleventh week, i.e., at the end of their lives. Foraging may be a workers' specialized task because it is performed by younger individuals, even if at low frequencies.

The number of workers performing the fungus garden care behavior was very similar between medium and small workers, with a drastic reduction of this activity from the tenth week of the experiment. A similar number of workers of both sizes performing this activity is contrasting because one would expect this behavior to be more frequent among small workers. These medium-sized workers are likely part of a garden-care specialist caste. Over the weeks, foraging was performed mainly by medium workers. Their size is compatible with plant material cutting, loading, and transportation.

Waste compartment tasks were performed throughout the workers' lives and were more frequent among the small workers during most of the experiment. It is noteworthy that the occurrence of waste management tasks was higher in the first two weeks. One would expect these tasks to be performed by older workers, considering that waste poses a greater risk of contamination with pathogens. One explanation is that not every microorganism in the nest refuse is hazardous to workers, or these workers have efficient defense mechanisms to inactivate potentially harmful microorganisms. Some pathogenic fungus, like the garden parasite *Escovopsis* (Bot et al., 2001), the fungal garden antagonist *Trichoderma viride* Pers. ex Gray (Lacerda et al., 2006; Ortiz & Orduz, 2001), and the entomopathogenic *Aspergillus flavus* Link ex Gray (Lacerda et al., 2006) and *Metarhizium anisopliae* (Metsch.) Sorokin (Hughes et al., 2004), for example, has been found in waste material. However, several others microorganisms found in those studies are not considered pathogenic to ants or the mutualistic symbiotic fungus. Leaf-cutting ants have

many strategies to prevent colony contamination, such as the production of effective antibiotics by metapleural glands, including some against entomopathogens (Poulsen et al., 2002), as well as actinomycetes associated with their bodies (Currie et al., 1999) that inhibit *Escovopsis* and entomopathogenic fungi (D'ângelo et al., 2016; Mattoso et al., 2012; Sen et al., 2009). In addition, there are behavioral strategies that help minimize contamination from waste, such as task sharing during waste transportation (Ballari et al., 2007; Hart & Ratnieks, 2002; Lacerda et al., 2006), allogrooming and self-grooming (Lacerda et al., 2013). Therefore, in our study, age polyethism was characterized by workers initially working in the garden and later in foraging activities; however, polyethism did not apply to waste-related tasks.

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Authors' contribution

FG Lacerda: investigation and writing.
TMC Della Lucia: conceptualization, methodology and writing.
L Souza-Souto: formal analysis and writing.
DJ de Souza: formal analysis and writing.

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