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# Ant Species That Remove Diaspores Alone Are More Efficient Removers

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**Abstract**

Secondary diaspore removal on the ground is an important ecosystem process. In this process, solitary foraging ants with larger body sizes are more efficient because they may remove more diaspores, faster and carry them at greater distances. Therefore, we sought to test the effects of the sizes of the morphological traits of ants, removal strategy, and nest distance on secondary diaspore removal, testing hypotheses related to the efficiency of this process. We evaluated the removal of artificial diaspores by ants in 15 areas of Cerrado *sensu stricto* (tropical savanna), collecting data on diaspore removal strategy (solitary or group), nest distance, diaspore discovery time, diaspore removal time, and the number of diaspores removed. Larger ants tended to remove diaspores alone and remove diaspores faster than smaller ones. Ants that removed diaspores alone removed more diaspores than ants that removed diaspores in groups. However, we did not find a linear relationship between ant size and diaspore removal. This is likely due to a limitation on, or a preference by larger ants for removing larger diaspores, while the smaller diaspores may have hindered manipulation or been less attractive to larger ants. Thus, the removal strategy was the best predictor of efficient diaspore removal performance, where the solitary foraging ants discover and remove diaspores quickly and remove more diaspores, mainly from the closest nests to the sampling point. However, the benefits (or not) of removing more diaspores still need to be evaluated.

## Introduction

Myrmecochory is an ecosystem function in which ants disperse approximately 11,000 species of myrmecochorous plants (Lengyel et al., 2010). Within this function, diaspore removal is the process in which ants transport diaspores, which is part of the seed dispersal. This is an asymmetric mutualism in which seeds of many angiosperm species are dispersed by a few ant species (Warren & Giladi, 2014). In general, ants are attracted by the high lipid content of seed elaiosomes for primary dispersal (Bas et al., 2007; Servigne & Detrain, 2008; Leal et al., 2014) and to the arils of diaspores on the ground for secondary dispersal (Pizo & Oliveira, 2000). Ants, along with birds, are the major secondary removers of fleshy diaspores falling on the grounds of Brazilian tropical

savannas (Campagnoli & Christianini, 2021; Christianini & Oliveira, 2010). These ants are typically large ants that forage solitarily and small ants that forage in groups, which are considered good and poor seed dispersers, respectively, due to their foraging characteristics and morphological traits (Giladi, 2006).

Large species of ants that forage solitarily, which are characterized by omnivorous or scavenging guilds, are considered to be high-quality dispersers because they disperse diaspores in greater quantity and at greater distances from parent plants (Giladi, 2006; Leal et al., 2014). Species that recruit many workers (generally small ants), such as omnivorous or granivorous ants, are considered to be poor-quality dispersers because they tend to disperse fewer diaspores over shorter distances (Hughes & Westoby, 1992;

Giladi, 2006; Leal et al., 2014). In addition, these ants often consume the aril, elaiosome, or even the seed at the site, performing few removals, making difficult the germination or even killing the seed embryo (Fernandes et al., 2018; Pizo & Oliveira, 2000). Conversely, large species are more attracted by elaiosomes with a greater content of some lipids because these components are also found in dead insects and in the prey of these ants (Hughes et al., 1994). So, this leads large ants that forage solitarily to discover and remove diaspores before the granivorous ants consume them (Hughes et al., 1994). Moreover, larger ants probably transport diaspores to the nest faster than small ants, because long-legged ants show the best performance in carrying loads (Nielsen et al., 1982; Espadaler & Gomes, 1996).

Conversely, the group-foraging ants, after finding resources and recruiting workers, continue to remove resources (Dornhaus & Powell, 2010). This behaviour can lead to faster removal of resources, due to the larger number of workers performing this function. On the other hand, although the solitary foraging ants do not recruit other workers, some ant groups, like some Ectatomminae ants, can show path fidelity, *i.e.,* the ants specialized in particular foraging zones around the colony (Pie, 2004). In this foraging type, the ants find the resources and return to the nest quickly, which can also increase the speed of removal of resources.

Some morphological traits of ants are considered useful to evaluate the effectiveness of ant performance in ecosystem processes and functions. Ants with larger bodies tend to carry more diaspores for greater distances (Ness et al., 2004), and probably discover and remove lipid-rich diaspores faster (Espadaler & Gomes, 1996; Hughes et al., 1994; Nielsen et al., 1982). This apparently is beneficial for myrmecochory, allowing increased plant dispersal and conquest of new territories (Bestelmeyer & Wiens, 2003; Ness et al., 2004). In addition, these ants often have larger legs, which indicate higher foraging speed and ease of movement on the ground (Feener Jr et al., 1988; Hurlbert et al., 2008; Silva & Brandão, 2010; Pearce-Duvet et al., 2011). Similarly, ants with larger mandibles can hold or carry larger food items (Weiser & Kaspari, 2006; Silva & Brandão, 2010; Gibb et al., 2015), which may be beneficial for diaspore dispersal because it allows these large ants to remove a wider variety of diaspores. However, larger ants tend to prefer to handle larger diaspores due to possible limitations in the manipulation of small diaspores and the lower attractiveness of them (Davidson, 1977; Takahashi & Itino, 2012; Anjos et al., 2018). Regarding orientation and search for resources, larger eyes may help in orientation (Hölldobler, 1980) and identification of food (Weiser & Kaspari, 2006; Gibb et al., 2015). Larger antennae scapes indicate greater chemosensory perception in foraging (Weiser & Kaspari, 2006; Silva & Brandão, 2010; Gibb et al., 2015).

Due to the apparent advantages already presented of larger ants in the removal of diaspores (Feener et al., 1988; Bestelmeyer & Wiens, 2003; Ness et al., 2004; Hurlbert et al., 2008; Pearce-Duvet et al., 2011), if plants can attract omnivorous or scavenging ants (larger ants), this can be advantageous for diaspore dispersal. However, the opposite has been observed, with smaller ants participating in most of the interactions with diaspores (Christianini & Oliveira, 2010; Anjos et al., 2020). In Brazilian savannas, the larger interactions between ants and fleshy diaspores falling on the ground happen with small ants (Christianini & Oliveira, 2010). However, large ants remove myrmechorous diaspores at a higher rate because they are rich in lipids (Hughes et al., 1994; Giladi, 2006). It is important to evaluate the effects of ants of different sizes and with different foraging strategies on diaspore removal from the savanna ground, as this is a key part of myrmecochory.

To evaluate these characteristics, we compared ant species to evaluate how morphological traits and solitarily or group foraging strategy affected secondary diaspore removal. We addressed the following questions and predictions: i) Can the diaspore removal foraging strategy be defined by the ant size? We predicted that larger species of ants would remove diaspores alone, while smaller species would remove diaspores in groups; ii) Do removal strategy, ant size and removal distance influence the time taken for diaspore discovery? We predicted that solitarily foraging ant species, larger ants with removal to greater distances, would discover diaspores faster; iii) Do removal strategy, ant size, and removal distance influence the speed of diaspore removal? We predicted that larger ants (solitary foraging ants with removal to greater distances) would remove diaspores faster; iv) Do ants remove diaspores increasingly faster? We predicted that after the first diaspore removal, the ants will remove diaspores quickly; v) Do removal strategy, ant size, and removal distance influence the number of diaspores removed? We predicted that larger ant species (solitary foraging ants with removal to greater distances) would remove more diaspores than smaller ants (group-foraging ants).

## Materials and Methods

### Study site

We collected the data on Cerrado *sensu stricto* vegetation (tropical savanna) within the Área de Proteção Ambiental do Rio Pandeiros (APA Rio Pandeiros). This protected area encompasses 393,866 hectares and is considered the largest Sustainable Use Conservation Unit in the state of Minas Gerais, Brazil. It is located in the north of the state (-15° 50′ S, -44° 76′ W) in a transitional region between the Cerrado and Caatinga biomes. The climate is semiarid, with well-defined seasonality. The temperature ranges from 9 °C in the cold (June and July) to 45 °C in the hot (October to January) season. The rainfall varies from 900 to 1250 mm throughout the year (Nunes et al., 2009). Field collections were performed in March 2016 during the hot and rainy season, the period of greatest ant activity in the Cerrado (Marques & Del-Claro, 2010).

**Sampling design and diaspore removal**

We sampled 15 areas of Cerrado spaced at least 500 m apart. In each area, 50 artificial diaspores were deposited at a sampling point 70 m from the edge of the Cerrado to avoid edge effects. The artificial diaspores were made of beads weighing 0.03 g and 2 mm in diameter, considered small, usually as myrmecochory diaspores (Anjos et al., 2020; Pizo & Oliveira, 2000), and an attractive paste composed of 75% hydrogenated vegetable fat, 7% casein, 5% maltodextrin, 4.8% fructose, 4.7% glucose, 3% calcium carbonate, and 0.5% sucrose. The beads simulated the solid part of the seed, while the attractive paste simulated the lipid-rich aril, a portion of the diaspore that is attractive to ants (see Raimundo et al., 2004). We used artificial diaspores to standardize sampling, since natural diaspores may vary in shape, size, and ripening stage, which could influence their interactions with ants. The use of artificial diaspores is a method employed to avoid problems with not finding enough natural diaspores at the time of the experiment and to standardize samplings, since natural diaspores may vary in shape, size, and ripening stage, which can influence their interactions with ants (Raimundo et al., 2004; Bieber et al., 2014; Rabello et al., 2015; Angotti et al., 2018; Rabelo et al., 2020, 2021).

We defined diaspore removal as the event in which the ants transported the artificial diaspores from the sampling point to the nest. The artificial diaspores were left exposed on the ground from 7 am to 10 am (period of high activity for most ant species). During this period, we recorded the time of diaspore discovery by the ants, speed of diaspore removal from the sampling point to the nest, distance from the sampling point to the nest, diaspore removal strategy (solitary or group), and number of diaspores removed. We marked the nest with diaspore removal and collected one ant per nest after experiments. We also collected individuals from the nests that performed the removals for subsequent species identification and measurement of morphological traits.

We assessed the removal strategy as whether ants transported the diaspores alone or in groups of workers. Solitary foraging occurred when only one ant carried the diaspore from the sampling point to the nest. Group foraging occurred when two or more workers jointly carried the diaspore to the nest. The groups of ants can vary in the number of individuals involved, both between different removal events and within the same removal event (during a removal, workers may join or leave the group, increasing or decreasing the number of ants).

Diaspore discovery occurred when one or more ants found and began to remove the diaspore. We defined discovery time as the number of seconds (s) from the beginning of the experiment (7:00 am) until ants began removal of an artificial diaspore. For each ant species, we calculated the mean discovery time in each of the 15 experimental areas.

The removal time was the time that the ants (with solitary behaviour or in groups) took to transport the artificial diaspore from the sampling point to the nest. For each ant species, we calculated the mean removal time in each of the 15 experimental areas.

To assess whether the removal time changed when the same nest performed more removals, we chose the nests where the ants performed at least three diaspore removals, and considered the removal times of first, second and last diaspore removal, regardless of how many removals the nest performed overall. We chose to perform the analyses only with species that had at least three replicates (species collected in at least three out of the 15 areas). For the analyses, we calculated the mean removal time of each ant species per area.

For each of the 15 areas, we calculated the arithmetic mean diaspore removals per species. In all cases, the sampling unit was the diaspore-removing ant species collected in each area. We only consider diaspore removal when the ants load the diaspores out of the sampling point to the nest. Other types of interactions between ants and diaspores, like ants that only interacted with diaspores on sampling point, which do not load diaspores until the nest, or that we do not achieve follow, were not considered in our study. We marked the nest with diaspore removal and collected one ant per nest after experiments.

**Species identification and morphological measurements**

The collected individuals were identified according to Palacio and Fernández (2003), Wilson and Hölldobler (2005), and Baccaro et al. (2015). The specimens were deposited in the reference collection of the Laboratório de Ecologia de Formigas at Universidade Federal de Lavras (UFLA). One individual from each nest was taken for morphological measurements. In species for which we sampled only one nest, only one individual was measured, whereas in species for which we sampled more than one nest, the mean of all individuals measured was calculated.

We measured the following morphological traits, which are considered important for diaspore removal: body size (Weber's length), which is the length from the anterior edge of the pronotum to the posterior edge of the propodeum (Weiser & Kaspari, 2006; Gibb et al., 2015); mandible size, which is the length from the mandibular insertion to the most external point (Weiser & Kaspari, 2006; Silva & Brandão, 2010; Gibb et al., 2015); eye width (Weiser & Kaspari, 2006; Gibb et al., 2015); scape size, which was measured by its length (Weiser & Kaspari, 2006; Silva & Brandão, 2010; Gibb et al., 2015); and leg size, which was the length of the femur added to the length of the tibia of the posterior metathorax (Weiser & Kaspari, 2006). All measurements were obtained in millimetres under a Zeiss Axio Zoom V16 microscope. The data is available in Supplementary Material.

**Data analysis**

First, we performed a correlation test on the morphological traits (Weber's length, mandible size, eye width, scape size, and leg size). The Spearman method was used due to the non-normality of the data. All morphological measurements (Weber's length, mandible size, eye width, scape size, and leg size) were correlated with each other (Table 1). To avoid problems of multicollinearity in the analyses, we chose only Weber's length as a predictor variable, considering that it synthesizes the other morphological traits in our data.

To test whether larger ants removed diaspores alone and smaller ants removed diaspores in groups, we performed a linear mixed-effects model (LMM) where the explanatory variable was the removal strategy (solitary or group), and the response variable was the body size (Weber's length in mm) and the fixed variable was species.

To test whether larger ants, solitary removal and larger distances removal is linked to discovery of diaspores and quickly diaspore removal, we constructed an LMM and a GLMM (generalized linear mixed model), respectively:

in LMM we are relating body size, removal strategy and removal distance (explanatory variables) with the discovery time (response variable), and the GLMM we relating them to removal time (response variable). In GLMM, we used the Poisson distribution family, but due overdispersion, we used quasi-Poisson distribution. In both models the fixed variable was species.

To test whether, after the first removal, the ants removed the diaspores faster, we constructed a GLMM to evaluate the effect of being the first, second, or last removal (explanatory variable) on removal time (response variable) and the fixed variable was species nest. We used the Poisson distribution family, but due overdispersion, we used quasi-Poisson distribution.

To test whether body size, foraging strategy and removal distance influenced the number of diaspores removed, we constructed a GLMM relating body size, removal strategy and removal distance (explanatory variables) with the proportion of diaspores removed (response variable)and the fixed variable was species. We used a quasi-Binomial distribution family because the values ranged from 0 to 1.

**Table 1.** Correlation coefficients in ants morphological traits.

|  | Weber's length | Mandible length | Eye width | Scape length | Leg length |
|---|---|---|---|---|---|
| Weber's length | - | 0.003 | <0.001 | <0.001 | <0.001 |
| Mandible length | 0.61 | - | 0.007 | <0.001 | <0.001 |
| Eye width | 0.93 | 0.56 | - | <0.001 | <0.001 |
| Scape length | 0.88 | 0.72 | 0.89 | - | <0.001 |
| Leg length | 0.86 | 0.69 | 0.91 | 0.97 | - |

p value above the dash and Spearman correlation below the dash.

All analyses were performed using the software R version 4.1.2 (2022). For the correlation analysis, we set $p < 0.05$ and Spearman's correlation coefficient $> 0.6$ as statistically significant; the correlations were tested with the Hmisc package (Harrell & Harrell Jr, 2019) and plotted with the corrplot package (Wei et al., 2017). For the LMMs and GLMMs with Poisson distribution family we used lme4 package (Bates et al., 2015). For the GLMMs with quasi-Poisson and quasi-Binomial we used MASS package (Venables & Ripley, 2002). To calculate Pseudo-$R^2$ we used jtools (Long, 2022) MuMIn package (Barton, 2013). We detected multicollinearity through VIF (variance inflation factor) and excluded variables that had VIF $> 4.0$. All graphs were generated using the ggplot2 package of R (Wickham, 2011).

**Results**

A total of nine species of diaspore-removing ants were identified. The subfamily Ectatomminae was the most frequent, removing artificial diaspores in 13 of the 15 sampled areas. Five ant species were classified as displaying a solitary foraging strategy in the 15 sampled areas: *Ectatomma opaciventre* (Roger, 1861), *Ectatomma edentatum* Roger, 1863,

*Ectatomma planidens* Borgmeier, 1939, *Ectatomma brunneum* Smith, 1858, and *Odontomachus haematodus* (Linnaeus, 1758). Four species displayed a group-foraging strategy in six sampled areas: *Blepharidatta conops* Kempf, 1967, *Pheidole jelskii* Mayr, 1884, *Pheidole capillata* Emery, 1906, and *Solenopsis tridens* Forel, 1911.

We indeed found that (i) larger ant species removed diaspores alone and smaller ant species remove in groups (df = 19; $\chi^2 > 33.566$; $p < 0.001$; Pseudo-$R^2$ = 0.98; Fig 1). On question (ii), we excluded ant size of model because detected multicollinearity through VIF (VIF = 6.004) and found that solitary remover ants (df = 18; $\chi^2$ = 15.974; $p < 0.001$; Pseudo-$R^2$ = 0.70; Fig 2) and ants which remove for shorter distances discovered diaspores faster (df = 18; $\chi^2$ = 5.384; $p = 0.020$; Pseudo-$R^2$ = 0.70; Fig 2). On question (iii), we excluded ant size of model (VIF = 8.747) and found that solitary remover ants removed diaspores faster (df = 19; $\chi^2$ = 52.274; $p < 0.001$; Pseudo-$R^2$ = 0.92; Fig 3), but the nest distance did not affect removal time (df = 18; $\chi^2$ = 0.345; $p = 0.556$; Pseudo-$R^2$ = 0.92). On question (iv), only one ant species was collected in more than three areas (replicate) and performed the three diaspore removals. In this case, we only made data analysis with *Ectatomma edentatum*

(larger species with solitary strategy). We found that the removal time decreases from the first to the last removal (df = 33; $\chi^2$ = 13.127; p = 0.001; Pseudo-R² = 0.76; Fig 4).
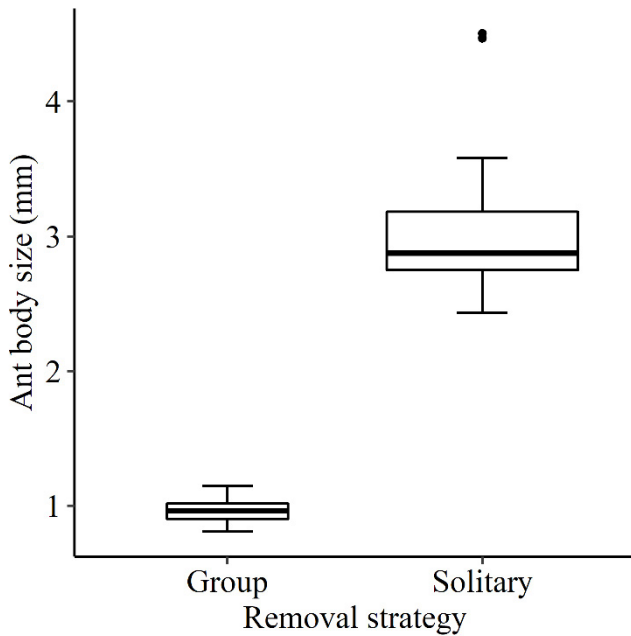
**Fig 1**. Smaller ants remove diaspores in groups, and larger ants remove diaspores alone. The *x*-axis indicates the removal strategy, and the *y*-axis indicates the ant body size in millimetres. The centreline of each boxplot indicates the median of all values, and the boxes indicate the first quartile (median of the values above the central median) and third quartile (median of the values below the central median). The vertical lines of each boxplot are the Tukey-style whiskers (1.5 × interquartile range). The circles above each boxplot are outliers.
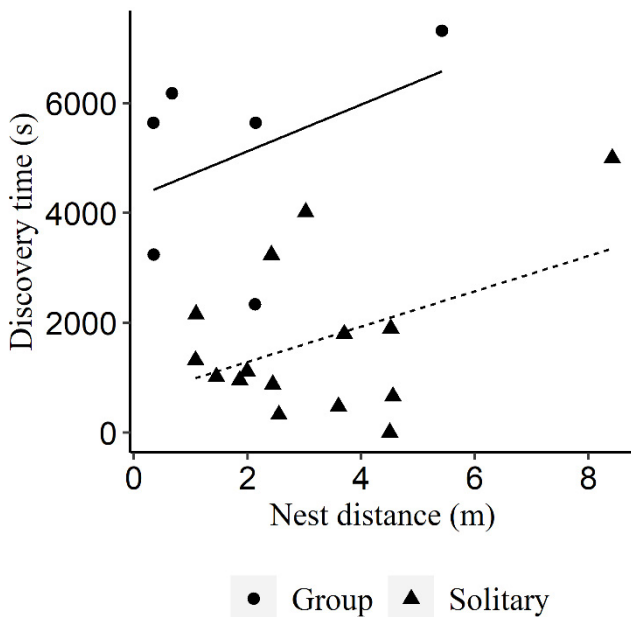
**Fig 2**. Ants with solitarily removal and ants with nests less distance discover diaspores faster. The *x*-axis indicates nest distance in meters (m) the removal strategy (solitary and group), and the *y*-axis indicates the diaspore discovery time in seconds (s). The circles and the line indicate group removal ants, and the triangles and dashed line indicate solitary remover ants.
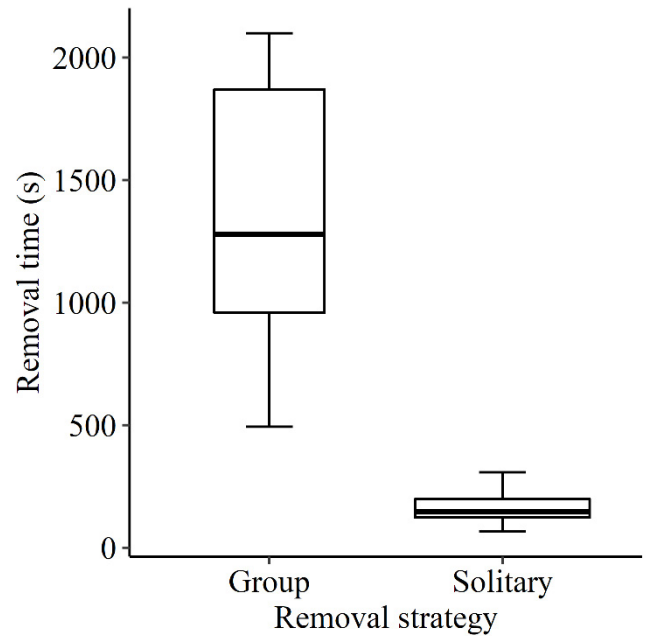
**Fig 3**. Solitary remover ants remove diaspores faster than group removal ants. The *x*-axis indicates the removal strategy, and the *y*-axis indicates the removal time in seconds. The centreline of each boxplot indicates the median of all values, and the boxes indicate the first quartile (median of the values above the central median) and third quartile (median of the values below the central median). The vertical lines of each boxplot are the Tukey-style whiskers (1.5 × interquartile range).
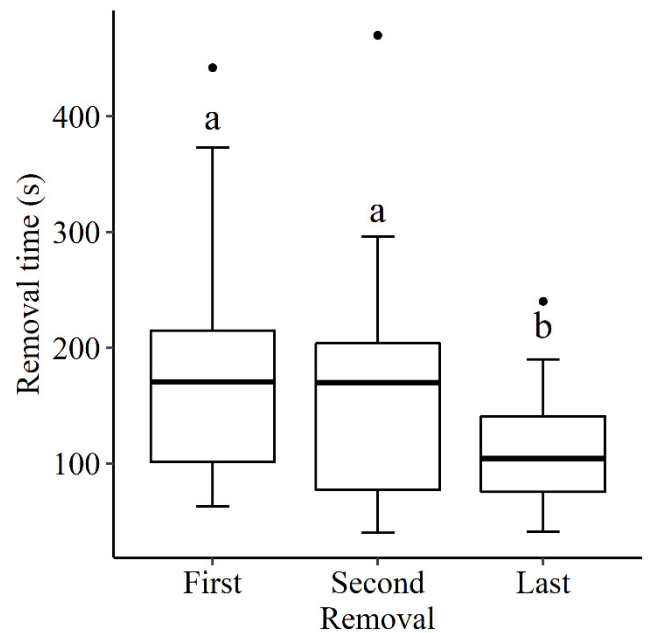
**Fig 4**. After the first diaspore removal, *Ectatomma edentatum* remove diaspores more quickly. The *x*-axis indicates which diaspore removal (first, second and last) and the y-axis indicate removal time in seconds (s). The centreline of each boxplot indicates the median of all values, and the boxes indicate the first quartile (median of the values above the central median) and third quartile (median of the values below the central median). The vertical lines of each boxplot are the Tukey-style whiskers (1.5 × interquartile range). The circle above the boxplot is an outlier.

Last, regarding objective (v), we found that solitary foraging ants removed more diaspores than group-foraging ants (df = 19; $\chi^2$ = 11.793; p < 0.001; Pseudo-$R^2$ = 0.39; Fig 5) and larger distance of nests decreases diaspore removal (df = 18; $\chi^2$ = 7.969; p = 0.004; Pseudo-$R^2$ = 0.39; Fig 5), but the ant size did not affect removal time (df = 17; $\chi^2$ = 1.888; p = 0.169; Pseudo-$R^2$ = 0.42).
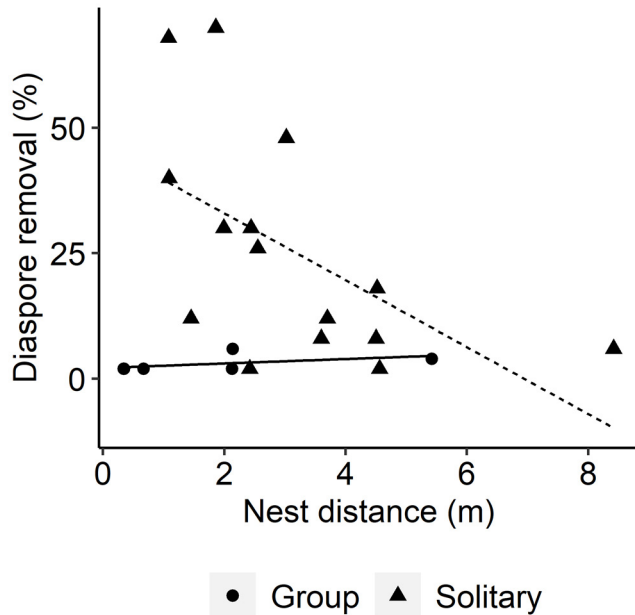


**Fig 5**. Ants with solitarily removal and ants with nests less distance (*x*-axis) remove more diaspores (*y*-axis). The number of diaspores removed is expressed as a percentage (number of diaspores removed by the species/total diaspores available at the sampling point*100). The circles and the line indicate group removal ants, and the triangles and dashed line indicate solitary remover ants.

**Discussion**

Overall, we found that solitary foraging ants were more effective in removing artificial diaspores. When considering the removal strategy, solitary remover ants (larger ants) discovered and transported diasporas quickly and removed more diasporas than ants with group foraging removal (small ants), showing a greater efficiency in this process, which can generate benefits for the myrmecochory function. *Ectatomma edentatum*, a solitary ant, removed the highest number of diaspores and with increased speeds, being more efficient. Moreover, ants with nests closer to diaspores, discovered and removed more diaspores, which can affect the dispersal distance.

Larger ants removed diaspores alone, and smaller ants removed them in groups. This probably occurred because larger mandibles allow ants to hold diaspores of different sizes more easily, and they have larger bodies and legs that afford them better locomotion on the ground (Silva & Brandão, 2010; Gibb et al., 2015; Feener et al., 1988), important characteristics for performing more efficient diaspore removal. Moreover, in addition to the morphological characteristics that can help these larger ants to remove alone, they have solitary foraging behaviour, providing

the search and removal of seeds alone (Gomes et al., 2009; Ostwald et al., 2018). In contrast, smaller ants are rarely able to hold and remove diaspores on their own, needing to recruit other workers to transport the resource. Our results corroborate Giladi (2006) and Leal et al. (2014), who separate ants into "good dispersers", composed of guilds of omnivorous or scavenging ants with solitary foraging, and "poor dispersers", which are guilds of granivorous ants that recruit many workers. We can draw a parallel between this classification and the finding that group removal ants (genera: *Blepharidatta*, *Pheidole* and *Solenopsis*) perform few removals and are often seen to consume the aril at the site (Pizo & Oliveira, 2000). These ants may in our study be considered "poor removers" since we only evaluated the characteristics of the diaspore removal process as part of the dispersal function. However, the small ants clean the seeds, decreasing the fungal attack and increasing the germination rate (Oliveira et al., 1995). Conversely, solitary remover ants (omnivorous or scavenging genera: *Ectatomma* and *Odontomachus*) removed more diaspores and were not observed to prey at the site, so they can be considered "good removers". In a complementary manner, *Ectatomma edentatum* was the species that most participated in interactions with diaspores in our study (≈60%). In addition to being a species with solitary removal, which discovers and removes resources faster, it is also considered a good disperser by Leal et al. (2014). However, it is important to notice that removal is part of seed dispersal process, and good-quality removal does not mean dispersal, as dispersal also depends on where the seed is deposited, how the ants manipulate the seed, and whether the seed germinates.

Still regarding the effect of guilds on diaspore removal, we find many interaction between omnivorous and scavenging ants which are attracted to rich lipid diaspores (Pizo & Oliveira, 2000; Bronstein et al., 2006), since our attractive paste contained 75% lipids, simulating fleshy diaspores, as done in other studies (Raimundo et al., 2004; Rabello et al., 2015; Angotti et al., 2018). More specifically, some lipids are even more attractive to scavenging ants (Hughes et al., 1994). This relationship between lipids and scavenging ants (large ants with solitary removal) occurs because these diaspore contents are the same as those found in insects that are prey to these ants (Hughes et al., 1994). These factors make the diaspores more attractive to scavenging ants, which may make it easier for them to find the diaspores faster than other guild or functional groups of group foraging ants. Moreover, the solitary foraging ants with nests closer to the diaspore, discovered diaspores faster than that with far nests, probably because they forage more around nests than in far areas.

In addition to discovering diaspores faster, solitary remover ants also removed the diaspores faster, probably because they had greater ease in removing the resources than smaller ants. Their larger legs provide greater ease and speed of locomotion (Silva & Brandão, 2010; Feener et al., 1988). Regarding the diaspore removal time to *Ectatomma edentatum* (solitary foraging ant), we found a decrease in removal time after the first transport, which suggests that a given nest does become more efficient at removal. *Ectatomma*

*edentatum* are Ectatomminae ants and can show path fidelity in particular foraging zones around the nest (Pie, 2004). So, the ants find the resources and return to the nest quickly and remove diaspores in a growing speed, increasing removal efficiency. In addition, in our study some nests with group foraging ants performed only one or few removals. This may be related to body limitations among smaller ants, preference for other resources, or interactions at the sampling point (*e.g.*, eat the diaspore at the sampling point).

We observed that solitary remover ants removed more diaspores than ants in groups, but there was no direct relationship between ant size and the number of diaspores removed. This finding suggests that solitary remover ants may have a size limit in the morphological traits that would let them perform such processes more efficiently. As we observed, larger ants with solitary removal remove more diaspores, probably because they have more easily to execute this function than smaller ants with group removal. However, ants are limited in body size and diaspore size. As observed by Anjos et al. (2018), there is a relationship between mandible size and diaspore size, and larger ants prefer to handle larger diaspores. In addition, Takahashi and Itino (2012) observed that large ants have difficulty handling small diaspores. In this sense, the standardization of the size of the diaspores we used, considered small, may have generated a limitation and a difficulty for some larger ants, such as *Ectatomma brunneum* and *Odontomachus haematodus*, which removed few diaspores. However, despite this limitation for larger size ants, when comparing solitary removers with group removers, ants that remove diaspores alone still have advantages and perform the removal process more efficiently due to the aforementioned characteristics that confer greater ease in finding and removing diaspores. Moreover, ants with nests closer to diaspores, removed more diaspores. In the same way for diaspore discovery, foraging more around nests than in far areas, facilitates to remove more diaspores. However, this can lead to a limitation on the distance of seed dispersal, where more diaspores are removed only for short distances, and this should be investigated.

We conclude that solitary remover ants are better diaspore removers than group removal ants. Solitary remover ants are ants with larger body sizes that discover and remove diaspores faster and in larger quantities. These ants are probably more efficient at secondary seed dispersal, as we observed that they remove more diaspores, probably decreasing the competition under the parent plant, but remove more for short distances, which can be a problem for dispersal, and we suggest this evaluation in future research. In addition, solitary remover ants find and remove diaspores quickly, and *Ectatomma edentatum*, the greater remover in our study, remove each time faster than the before one, decreasing the diaspore exposure time to fungi, which are likely to make the diaspores unfeasible (Oliveira et al., 1995), or to vertebrate predators and granivorous ants (Christianini & Oliveira, 2010). Smaller ants with group removal discover and remove diaspores slowly and in smaller quantities. They perform fewer dispersals (Giladi, 2006) for shorter distances (Ness et al., 2004) and

are still often seen eating the attractant at the site, decreasing dispersal (Christianini & Oliveira, 2010), or eating the seed, killing the embryo. Thus, although it has been an assumption that ant morphological traits are an indication of an efficient diaspore removal performance, this is not always true, since the morphological trait evaluated by us and by most studies on this topic – ant body size – were not the best predictor of diaspore removal, but rather the removal strategy was the best predictor. Moreover, these results can change due to the chemical composition of diaspores. The larger ants in our study were probably attracted by lipid composition of diaspores, but the chemical composition influences the identity and groups of ants that interact with diaspores (Campagnoli & Christianini, 2021; Pizo & Oliveira, 2001).

## Authors' Contribution

IW: Conceptualization, Methodology, Validation, Formal analysis, Data Curation, Writing-Original Draft, Writing-Review & Editing, Visualization.

MAR: Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing-Review & Editing, Supervision, Project administration.

MAA: Conceptualization, Methodology, Validation, Investigation, Writing-Review & Editing.

CRR: Conceptualization, Methodology, Validation, Resources, Writing-Review & Editing, Supervision, Project administration, Funding acquisition.

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## Supplementary Material

**Table S1**. Sampling areas, species sampled, and variables collected: sample (sampled areas - 1 to 15); specie (ants species - Hymenoptera: Formicidae); ML (Mesosomal or Weber's length - millimeters - mm); MandL (Mandible length - millimeters - mm); EL (Eye length - millimeters - mm); SL (Scape length - millimeters - mm); FL (Leg length - summing the femur and tibia length of posterior metathorax leg - millimeters - mm); strategy (foraging strategy - solitary or group); discovery (discovery time of diaspores - seconds - s); time_r (removal time of diaspores - seconds - s); diaspore_removal (artificial diaspores removal by ants - Hymenoptera: Formicidae).

| Sample | Species | ML | MandL | EL | SL | FL | Strategy | Discovery | Distance | Time_r | Diaspore_removal |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Ectatomma edentatum* | 2.549 | 0.909 | 0.291 | 1.481 | 4.075 | Solitary | 660 | 4.56 | 289 | 1 |
| 1 | *Pheidole jelskii* | 0.892 | 0.360 | 0.108 | 0.721 | 1.650 | Group | 3240 | 0.35 | 1960 | 1 |
| 2 | *Ectatomma opaciventre* | 4.506 | 7.782 | 0.475 | 2.661 | 7.260 | Solitary | 4.11 | 4.50 | 198 | 4 |
| 2 | *Pheidole jelskii* | 1.146 | 0.442 | 0.126 | 0.808 | 1.560 | Group | 7320 | 5.42 | 960 | 2 |
| 3 | *Ectatomma planidens* | 2.433 | 0.509 | 0.224 | 0.759 | 1.276 | Solitary | 2160 | 1.09 | 128 | 20 |
| 4 | *Ectatomma edentatum* | 2.744 | 0.941 | 0.267 | 1.579 | 4.023 | Solitary | 880 | 2.44 | 177 | 15 |
| 5 | *Ectatomma edentatum* | 2.993 | 1.268 | 0.392 | 1.515 | 4.700 | Solitary | 960 | 1.86 | 101 | 35 |
| 6 | *Ectatomma edentatum* | 3.064 | 1.311 | 0.435 | 1.578 | 3.995 | Solitary | 1320 | 1.08 | 65 | 34 |
| 7 | *Ectatomma edentatum* | 2.875 | 1.130 | 0.348 | 1.616 | 4.303 | Solitary | 1120 | 1.99 | 116 | 15 |
| 8 | *Ectatomma edentatum* | 2.489 | 1.182 | 0.364 | 1.637 | 4.194 | Solitary | 1020 | 1.45 | 131 | 6 |
| 9 | *Ectatomma edentatum* | 2.858 | 1.086 | 0.313 | 1.519 | 3.820 | Solitary | 1900 | 4.52 | 146 | 9 |
| 9 | *Pheidole jelskii* | 1.024 | 0.391 | 0.122 | 1.044 | 2.260 | Group | 2340 | 2.13 | 960 | 1 |
| 10 | *Ectatomma edentatum* | 2.818 | 1.050 | 0.323 | 1.557 | 4.095 | Solitary | 330 | 2.55 | 156 | 13 |
| 11 | *Ectatomma edentatum* | 2.758 | 1.048 | 0.302 | 1.612 | 3.950 | Solitary | 480 | 3.60 | 280 | 4 |
| 12 | *Odontomachus haematodus* | 3.221 | 1.017 | 0.262 | 1.527 | 2.924 | Solitary | 1800 | 3.70 | 123 | 6 |
| 12 | *Ectatomma opaciventre* | 4.471 | 1.828 | 0.537 | 2.568 | 7.759 | Solitary | 5000 | 8.42 | 307 | 3 |
| 13 | *Odontomachus haematodus* | 3.144 | 0.968 | 0.275 | 1.557 | 3.069 | Solitary | 3240 | 2.42 | 200 | 1 |
| 13 | *Ectatomma brunneum* | 3.581 | 0.905 | 0.359 | 1.137 | 3.510 | Solitary | 4020 | 3.02 | 123 | 24 |
| 14 | *Pheidole capillata* | 0.807 | 0.451 | 0.099 | 0.702 | 1.670 | Group | 5640 | 2.14 | 1601 | 3 |
| 15 | *Blepharidatta conopsi* | 0.994 | 0.330 | 0.073 | 0.639 | 1.450 | Group | 5640 | 0.34 | 493 | 1 |
| 15 | *Solenopsis tridens* | 0.930 | 0.276 | 0.072 | 0.656 | 1.390 | Group | 6180 | 0.67 | 2100 | 1 |

**Table S2**. Nests sampled in each area, species sampled (only *Ectatomma edentatum*), removal performed (first, second, and last), and removal time (s).

| Nest | Species | Time_r | N_removal |
|------|---------|-------:|-----------|
| A4N1 | *Ectatomma edentatum* | 373 | First |
| A4N1 | *Ectatomma edentatum* | 178 | Second |
| A4N1 | *Ectatomma edentatum* | 149 | Last |
| A4N2 | *Ectatomma edentatum* | 63 | First |
| A4N2 | *Ectatomma edentatum* | 40 | Second |
| A4N2 | *Ectatomma edentatum* | 41 | Last |
| A5N1 | *Ectatomma edentatum* | 161 | First |
| A5N1 | *Ectatomma edentatum* | 187 | Second |
| A5N1 | *Ectatomma edentatum* | 94 | Last |
| A6N1 | *Ectatomma edentatum* | 87 | First |
| A6N1 | *Ectatomma edentatum* | 80 | Second |
| A6N1 | *Ectatomma edentatum* | 58 | Last |
| A7N1 | *Ectatomma edentatum* | 66 | First |
| A7N1 | *Ectatomma edentatum* | 57 | Second |
| A7N1 | *Ectatomma edentatum* | 71 | Last |
| A7N2 | *Ectatomma edentatum* | 196 | First |
| A7N2 | *Ectatomma edentatum* | 140 | Second |
| A7N2 | *Ectatomma edentatum* | 97 | Last |
| A7N4 | *Ectatomma edentatum* | 180 | First |
| A7N4 | *Ectatomma edentatum* | 162 | Second |
| A7N4 | *Ectatomma edentatum* | 112 | Last |
| A8N1 | *Ectatomma edentatum* | 106 | First |
| A8N1 | *Ectatomma edentatum* | 69 | Second |
| A8N1 | *Ectatomma edentatum* | 77 | Last |
| A9N1 | *Ectatomma edentatum* | 242 | First |
| A9N1 | *Ectatomma edentatum* | 296 | Second |
| A9N1 | *Ectatomma edentatum* | 240 | Last |
| A9N2 | *Ectatomma edentatum* | 206 | First |
| A9N2 | *Ectatomma edentatum* | 470 | Second |
| A9N2 | *Ectatomma edentatum* | 190 | Last |
| A10N1 | *Ectatomma edentatum* | 120 | First |
| A10N1 | *Ectatomma edentatum* | 195 | Second |
| A10N1 | *Ectatomma edentatum* | 130 | Last |
| A10N2 | *Ectatomma edentatum* | 442 | First |
| A10N2 | *Ectatomma edentatum* | 230 | Second |
| A10N2 | *Ectatomma edentatum* | 138 | Last |