



Behavioral and morphological traits influencing variation in task performance of *Camponotus vagus* ants

G. Trigos-Peral¹ · I. E. Maák^{1,2} · P. Ślipiński¹ · M. Witek¹

Received: 25 April 2023 / Revised: 20 September 2023 / Accepted: 25 September 2023 / Published online: 30 October 2023
© The Author(s) 2023

Abstract

The colony performance of social insects is supported by an efficient allocation of tasks among workers in the colony. The division of labor among ant workers is linked to age and caste polyethism, however, only a few studies have shown the relationship between workers' behavioral variation and task performance. In this study, we investigated the task syndrome by testing the relationship between behavioral traits with task performance and *location switching* (switching the performance of tasks from inside to outside the nest) in the same-age workers of *Camponotus vagus* ants. We also investigated the division of labor in workers with and without *location switching* by checking if each caste is characterized by specific behavioral traits or tasks. Moreover, we checked whether the difference in the worker's body size and its lifespan can influence task performance. Our results support the existence of task syndrome in ant workers by showing how individuals with specific behavioral traits are more likely to perform specific tasks within the colony. Moreover, the time of *location switching* was correlated with the tasks performed by the workers (*brood care*, *selfgrooming* and *walking*) and their behavioral traits (*aggressiveness* and *total distance*). In addition, worker size and lifespan are shown to influence task performance by the workers. Altogether, our study underpins the relationship between the behavioral traits and the task allocation and performance of workers within an ant colony.

Keywords Division of labor · Foraging · Brood care · Social interaction · Task switching · Task syndrome

Introduction

The foundation of ant ecological success is their self-organization, reflected as the division of labor, which in broad terms leads to the separation of a small number of individuals ensuring colony reproduction from a much higher number of individuals fulfilling other tasks. The division of labor in colonies of social insects can be influenced by the worker's genotype, size (morphological polyethism), age (temporal polyethism) or can be driven by task demand (foraging for work) within the colony (Tripet and Nonacs, 2004, Corona et al. 2013). In age polyethism, older workers perform tasks outside the nest, such as foraging, whereas younger workers conduct tasks within the nest such as

cleaning or queen/brood nursing (Bernadou et al. 2015; Giehr et al. 2017; Iwasa and Yamaguchi 2020). Some studies show also that individual life expectancy can play an important role in task performance; individuals with shortened life expectancy perform more risky tasks (e.g., foraging) (Woyciechowski and Kozłowski 1998; Moroń et al. 2008). The individual task performance of workers can be also modulated by a combination of different factors such as the level of juvenile hormone, fat content or individual experience (Ravary et al. 2007; Jeanson and Weidenmüller 2014; Norman and Hughes 2016; Bernadou et al. 2020). However, the task that individuals perform is not only determined by the characteristics of that individual but also by a set of interactions among individuals and their environments (Gordon 2016). As a response to social interactions and external stimuli, inter-individual differences in task allocation may arise through changes in the response thresholds of workers, resulting in prompt (low threshold) or less likely (high threshold) participation in a specific task (Beshers and Fewell 2001; Gordon 2019). The success of colonies of the same species inhabiting the same habitat may vary due to

✉ G. Trigos-Peral
getriral@gmail.com; gtrigos@miiz.waw.pl

¹ Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw, Poland

² Department of Ecology, University of Szeged, Szeged, Hungary

differences in their response to changing conditions (Gordon 2013; Maák et al. 2021). These differences may arise due to environmental conditions (external factors) that can alter the interaction network of workers, but also because of internal factors that determine the effectiveness of the workers (task performance) and influence colony productivity (Gordon 2019). Behavioral studies have shown that the basis of these inter-colonial behavioral differences is the so-called colony personality, defined as the combination of worker personalities and social interactions influenced by the environment (Blight et al. 2016; Carere et al. 2018). Nowadays, several studies have also demonstrated the existence of behavioral differences among ant workers of the same colony (Cerdá and Retana 1992; Sih et al. 2004; Lenoir et al. 2009; Cronin 2015; Maák et al. 2021), using different terminology for this phenomenon such as “idiosyncrasy” or “behavioral type” of workers. Such differences in behavior on the individual level can determine the effectiveness of workers during task performance. The “task syndrome” (Loftus et al. 2021), known as the relationship between the behavioral tendencies of the workers and the tasks they perform in the nest, can play a major role in the division of labor, and as a result, in the colony success (e.g., Modlmeier and Foitzik 2011). Recently, the variation of behavior among individuals within a colony has been treated as one of the key factors responsible for understanding the division of labour (Pamminger et al. 2014; Maák et al. 2020). However, several questions remain unanswered due to the complexity of this topic, which encompasses a large number of disciplines (Beekman and Jordan 2017), and there are only a few empirical studies showing the interplay between personality and the division of labor in social insect colonies (Máak et al. 2020; Loftus et al. 2021).

In this study, we investigate which individual traits of ants are involved in the task syndrome, which describes a consistent relationship between the behavioral traits of individuals and the tasks performed by them. Our research is an attempt to provide new insights in the field of behavioral ecology by studying the relationship among individual behavioral traits and the task performance of *Camponotus vagus* workers, but also the consistency of the task performance and behavioral traits along the ant worker life. Our focal species, by having polymorphic workers, allows us to trace the effects of morphological differences on task syndrome. Moreover, by choosing workers of the same age and colonies of the same size, we could also exclude some parts of the external factors affecting individual behavior and task performance and focus more on the effects of individual traits.

To decipher the relationship between behavioral traits (*aggression*, *boldness*, *total distance* and *total meandering*) and tasks performed by workers, we followed the task allocation of individual workers from the callow stage until their death. Our main hypotheses are that the behavioural

and morphological traits of individual workers and their life expectancy influence their task performance inside the colony, including task switching from intranidal tasks to foraging. We expected that (a) workers with higher exploration and locomotory skills become foragers earlier in life, (b) morphological traits of workers affect their behavior and task performance, and (c) after emergence, individuals already show a series of behavioral traits that are consistent across the time.

Materials and methods

Colony housing

For our study, we collected 10 entire colonies of *Camponotus vagus* from the field in the late spring of 2017 and 2018 near Ásotthalom village, Hungary (N 46.216217, E 19.784360). The colonies were later taken to the laboratory and kept in plastic containers (43 × 33 × 21.4 cm) at room temperature (~23 °C) in a natural light/dark cycle. To easily select and mark the newly emerged individuals, each original colony was checked twice per week for new pupae. Then, all new pupae were placed into a new plastic box (20 × 30 × 10 cm) together with 10 workers from the original colony. In this way, we could observe pupal development and trace and handle the emerging workers. Freshly hatched workers were individually marked with colored Art Deco enamel paint markers. Before marking, we waited until they reached a darker cuticular color (2–3 days), a signal of cuticular hardening. The day after marking, we placed the marked individuals in an artificial Ytong nest (20 × 20 × 5 cm) with a glass lid. Each Ytong nest was covered with a red foil to imitate the dark conditions inside a nest and connected with a silicone tube to a foraging arena (10 × 4 × 5 cm), where water was provided ad libitum and food (frozen killed crickets, *Drosophila* sp., honey-water) was provided three times per week for every colony. This setup allowed us to easily scan the behaviors of marked workers inside the Ytong nests. Marked individuals were kept together with 70 workers (50 foragers and 20 intra-nidal workers selected from the original colony by the position they were found inside the nest) and 10 larvae taken from their original colonies. After every 10–14 days, in every colony, we checked the number of workers and brood, and if necessary, they were replenished to the original number.

Worker task performance inside the nests

One part of the study focused on the observation of tasks performed by marked ants inside and outside (foraging area) of the Ytong nests. Observations started 10–12 days after the ants were introduced to the nest, and the tasks carried out

by each individual were recorded by visual scanning (e.g., Bernadou et al. 2015). During each scan, the location of the marked ants (inside/outside the nest) was also recorded. The marked ants were scanned once per day (between 9 am and 3 pm), five times per week (from Monday to Friday). Scanning lasted until the death of all paint-marked workers. The number of scans varied between 16 (min) and 58 (max), depending on the lifespan of each individual.

We defined task performance as the different assignments that workers performed for colony maintenance. The tasks carried out by the workers observed during this part of the study were categorized as *antennation*, *trophallaxis*, *allogrooming*, *brood care*, *inactivity*, *walking*, *selfgrooming* (definitions in Table S1). Moreover, for each marked worker, we recorded the lifespan (days of life of the worker since emerging from the cocoon) and traced the time of *location switching*, which we considered as the number of days the worker spent in the nest until it performed a task outside the nest for the first time. Based on this information, we categorized every worker into two main castes: switching and not-switching workers. Moreover, taking into account the behavioral maturation of the workers (Richardson et al. 2021), switching workers were also separated into two groups: nurse stage (for the period before leaving the nest) and forager stage (for the period after carrying out tasks outside the nest). On the other hand, not-switching workers were considered those who were recorded exclusively inside the nest and were never seen in the foraging arena.

Worker size

The marked workers used in the study were measured to check whether worker size is linked to the studied groups of tasks and behavioral traits. As in many cases, the bodies of dead workers were cut by their nestmates, we could not take direct measurements of these individuals. Therefore, we measured the ants by analyzing the videos and capturing a photo of an ant when its position allowed the proper visualization of their head. We then measured the head width (in mm) by using the ImageJ software (version 1.53e, <http://imagej.nih.gov/ij>).

Behavioral traits of workers

We studied the behavioral traits of individuals by conducting the following behavioral assays: *aggression*, *boldness*, *total distance* (as an estimation for *exploration*) and *total meandering* of a worker's walking path. Before being used in the behavioral assays, the marked workers were separated and put into plastic boxes (20 × 13 × 5 cm) together with a piece of wet sponge covered by a notched flowerpot saucer to provide an artificial shelter for ants. Marked individuals

stayed inside these boxes one hour prior to the assays and they were placed back into their Ytong nests afterwards. The selected workers were tested for all behavioral assays on the same day with a half an hour break between the assays. Behavioral assays were conducted once per month for each individual, with the first one being performed when the focal ant was one month old. Every behavioral trait assay was repeated at least two times per each studied ant (depending on the life expectancy of the worker).

Aggression assay

The aim of this assay was to determine the differences in aggression among marked workers towards corpses of *Formica polyctena*, an aggressive territorial rival species. The experiment was conducted in a plastic box (20 × 13 × 5 cm) with its walls coated with paraffin to prevent ants from escaping. Before starting the assay, we placed the focal ant inside the box and allowed it to habituate for a period of 5 min. After this time, we placed a corpse (proxy of live individuals) of *F. polyctena* worker inside the box. Corpses were freshly killed by freezing (−20 °C) and removed 5 min prior to the assays to avoid changes in their CHC (cuticular hydrocarbon) profile, which might deter the response of the tested ants (see e.g., Choe et al. 2009). In the assay, we considered the first reaction of the marked ant towards the corpse to evaluate the aggression level. Four categories of behaviors were observed: *fleeing* (rapid movement of the focal ant in the opposite direction), *antennation* (touching the corpse with the antennae), *mandible opening* (opening the mandibles in the presence of the corpse), *biting* (biting the corpse with the mandibles). We assigned a score to each behavior according to the increase in the aggression towards the proxy of the live individual: *fleeing* = 0, *antennation* = 1, *mandible opening* = 2, and *biting* = 3. To avoid any influence on the behavior of the tested ant due to the presence of pheromones released by the previously tested worker, we changed the test-boxes after every assay and cleaned them with alcohol.

Boldness assay

The aim of this assay was to test the boldness of the marked individuals. This assay was conducted in a plastic box (34.5 × 42 × 26 cm) and the focal ant was placed in a dark falcon tube (25 ml) that was closed afterward with a plastic lid and introduced in the middle of the arena. The tested worker was kept inside the tube for 2 min to habituate to the new place. Afterward, the lid was removed and a 2 min observation was conducted to record the time when the ant left the tube (used as an indicator of *boldness*). Ants were

considered bolder when they left the tube earlier. The plastic boxes were changed and cleaned with alcohol after each test.

Locomotion assay

The aim of this assay was to test the exploration skill and movement pattern of marked individuals. This assay was conducted in a Petri dish with a diameter of 19 cm, functioning as an arena. Before conducting this assay, the marked ant was placed for 2 min into a cylinder coated with flouon in the middle of the arena. This allowed the workers to calm down and habituate to the new place. After this period the cylinder was removed and a 3 min video recording was made. The camera (Microsoft lifecam studio) was placed 20 cm above the Petri dish, along with two light sources (one at each side of the camera) to homogenize the illumination of the arena. The locomotory parameters were measured by analyzing the videos with the help of EthoVision (EthoVision® XT v. 12; Noldus Information Technology, 2016) software. For the statistical analyses, two parameters were used: *total distance* traveled (centimeters walked by the focal ant during the 3 min observation period) and *total meandering* (changes in the direction of the focal ant's path measured in deg/cm). The Petri dishes were changed and cleaned with alcohol between subsequent assays.

Statistical analyses

Task performance

We performed two separate principal component analysis (PCA) for the different individual tasks (*antennation*, *trophallaxis*, *allogrooming*, *brood care*, *inactivity*, *walking* and *selfgrooming*) performed by each marked worker. The first PCA was applied on the matrix of the proportion of each task to the total number of tasks performed by each individual till the first task was performed outside of the nest (*location switching*). The second PCA was applied to the matrix of the proportion of tasks performed after the *location switching* by using only workers that switched location (switching workers—forager stage). Later, the differences in task performance between not-switching workers and switching workers were tested by conducting PERMANOVA (Euclidean distance) analyses on the matrix of the tasks performed by each individual during the observation.

Relationship between task performance and worker caste

The differences in the task performance among the studied workers were graphically represented in separated heatmaps for not-switching and switching workers, in which we included the frequencies of the performance of the different tasks from the beginning of the observation until the death of the ant workers. In the switching workers, we generated two different heatmaps corresponding to the number of tasks performed by the switching workers at the nurse stage and at the forager stage.

Differences in the number of task performances between not-switching and switching workers were tested by carrying out GLMMs (binomial error, maximum-likelihood fit). In the formula, the proportion of the number of each task to the total number of tasks performed by the individual was used as a response variable, whereas the caste (not-switching or switching workers) was used as an explanatory variable.

Consistency of task performance by switching workers over time

We tested the consistency in task allocation along the total lifetime of the switching workers by testing for differences in the task performance between the periods before and after the *location switching* by using a GLMM (binomial error, likelihood fit). In the model, the proportion of each task to the total tasks performed by the workers in the periods before and after *location switching* was used as a response variable, the days passed from the first observation to the *location switching* as an explanatory variable.

Task syndrome (association between task performance and behavioral traits)

We tested the existence of task syndrome by establishing the relationship between the behavioral profile of the workers and the tasks performed by them. To do this, we identified the existence of different behavioral profiles by performing a PCA on the matrix of behavioral traits of the workers. Later, we carried out a separate GLMM (binomial error, maximum-likelihood fit) per each task. In the models, the proportion of the number of times that a task was performed to the total number of tasks performed by the workers was used as a response variable, the coordinates of the PC1 and PC2 were used as explanatory variables.

The effect of behavioral traits on location switching

We performed GLMMs (Poisson distribution, maximum-likelihood fit) to test whether the *location switching* was linked to the behavioral traits of the workers. In the models, the proportion of days of lifespan passed from the first observation until *location switching* was used as a response variable. For this test, we only used the data obtained from the *location switching* workers. In the model, the behavioral traits (average scores of each trait for boldness and aggressiveness, and the scores obtained in the locomotion assay) were used as explanatory variables.

Worker size and lifespan influence on the task performance and behavioral traits

We checked whether the lifespan and the size of the workers are linked with their task performance, behavioral traits and *location switching*. Due to the differences in the measurement scale (lifespan—days, worker size—millimeters), the variables were scaled before performing the analyses. Separate models were used to test the effect of different variables on the proportion of the different tasks to the total number of tasks observed (GLMM; binomial error, maximum likelihood fit), on the behavioral traits (LMM, maximum likelihood fit), and on the *location switching* (GLMM; binomial error, maximum likelihood fit). In the models, the lifespan (number of days since the worker emerged until its death) and the worker size (head width, measured in mm) were used as explanatory variables.

Repeatability of the behavioral traits

The repeatability across the first two sessions of the behavioral trait analyses (N = 42 in the behavioral assays, and N = 39 in the locomotion assay) was assessed for all individuals tested in the behavioral assays (including both not-switching and switching workers) with intra-class correlations (Lessells and Boag 1987) by using LMM calculations (Nakagawa and Schielzeth 2010). Repeatability is used as the measurement of behavioral consistency and describes personality (Bell et al. 2007).

All statistical analyses were performed in the R Statistical Environment (R Core Team 2021). The PCA was performed using the PCA function from the *FactoMineR* package (Lê et al. 2008). The repeatability was calculated with the rpt function (rptR package, Stoffel et al. 2017). We assessed 95% confidence intervals (CI95%) by 1000 bootstraps and *p* values by 1000 permutations (alpha level = 0.05). The *ggplot* function was from the *ggplot2* package (Wickham 2016), and the heatmap was generated with the *reshape2* (Wickham 2007), *plyr* (Wickham 2011), *scales* (Wickham

and Seidel 2022) and *lattice* (Sarkar 2008) packages. The *fviz_pca_biplot* function from the *factoextra* package (Kassambara and Mundt 2016) was used in addition to the *ggplot2* functions to perform the graphical visualization of the PCA. PERMANOVA was performed using the *adonis2* function from the *vegan* package (Oksanen et al. 2019). LMMs were performed using the *lmer* function, and GLMMs were performed by using the *glmer* function from the *lme4* package (Bates et al. 2013). We checked for overdispersion by using the *testDispersion* function from the DHARMA package (Hartig and Lohse 2020). If necessary, the variables were log-transformed prior to the analyses to meet the normality and homogeneity of variances. Since locomotory variables were measured on different scales, these variables were scaled before using them in our models. In all the GLMM analyses, the individual ID was used as a random factor.

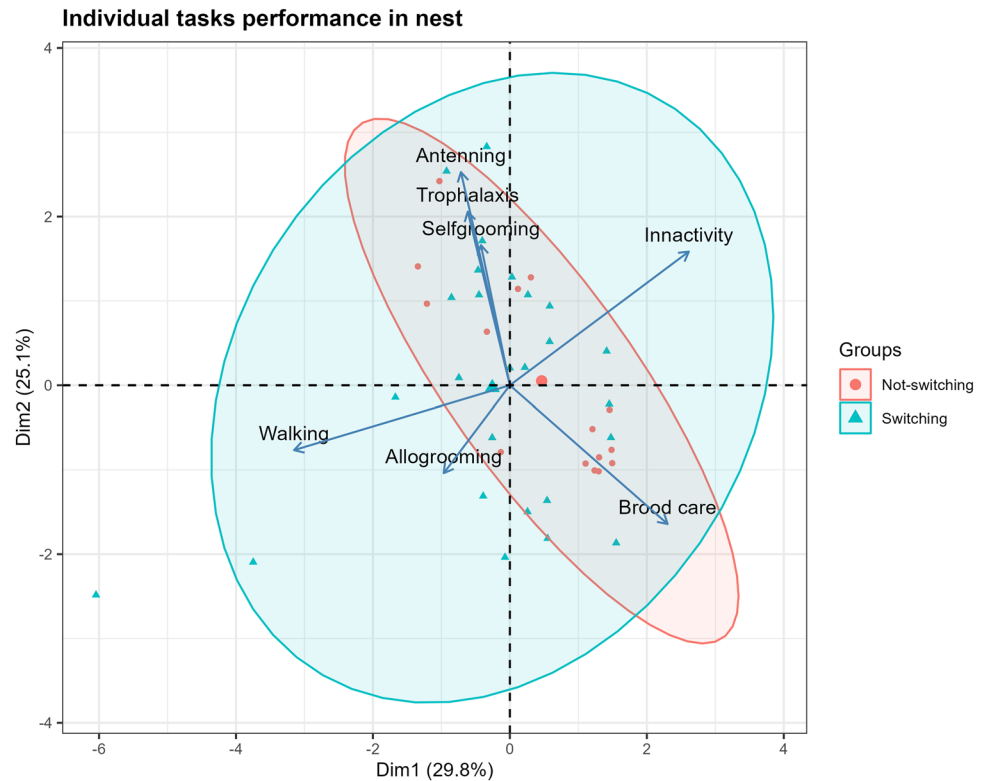
Results

Task performance

A total number of 42 individually marked ants were observed and tested during a period of three months. Workers showed different trends in both the performance of tasks as well as in the behavioral traits. Moreover, workers also differed in their lifespan and body size, (see Table S1). *Location switching* was never observed in 35.7% of the marked workers called as not-switching individuals, whereas in 64.3% of workers (switching) *location switching* occurred in some moment of their lifespan. The heatmap representation of the tasks performed by workers showed differences in task performance among individuals between the two castes (switching and not-switching) (Supplementary Fig. 1). The heatmap representation also showed differences in the task performance of nurse and forager stages of the switching workers.

The PCA was applied to the tasks performed by the ants during the time spent within the nests before the location switching was carried out by using all marked, 42 ant workers. The first principal component axis (PC1) explained 29.8% of the variability of the studied tasks, whereas the second one explained 25.08% (PC2) (Fig. 1). Based on PC1, brood care, a task performed exclusively within the nest, was positively associated with inactivity and negatively associated with walk. PC2 showed a negative significant association of brood care with *antennation*, *trophallaxis* and *selfgrooming*. Task performance differed between the two castes, not-switching and switching workers (PERMANOVA: $F_{1,41} = 6.84, p = 0.002$).

Fig. 1 PCA graph of the tasks carried out by the workers within the nest before location switching occurred. Ellipses (95% confidence) define the tasks carried out by each worker caste



The GLMM results on the tasks performed by the workers during the whole period of observation show that not-switching workers were more prone to carry out *brood care* ($z = 4.79$, $p < 0.001$) and *selfgrooming* ($z = 2.54$, $p = 0.011$), but also *walked* less ($z = -2.56$, $p = 0.011$) than switching workers. Moreover, the performance of the other studied tasks was not different between castes (Fig. 2).

Based on the PCA performed on the matrix of tasks performed only by switching workers during the whole time of observation, 28.4% of the variation among the traits was explained by PC1 and 22.1% by PC2 (Supplementary Fig. 2). Following the trend in previous analyses, PC1 showed to be segregated into two groups: one in which more interactive tasks were associated (*trophallaxis*, *allogrooming*, *antennation* and *walk*) and a second group in which less active tasks (*brood care* and *inactivity*) were associated.

Consistency of task performance by switching workers over time

The tasks performed by the switching workers varied from before to after the *location switching*. During the nurse stage, workers performed more *brood care* ($z = 5.05$, $p < 0.001$), less *trophallaxis* ($z = -1.95$, $p = 0.050$) and less *walk* ($z = -3.54$, $p < 0.001$) than during the forager stage (Fig. 2). No differences were found in the performance of the

other tasks between the periods before and after the *location switching*.

Relationship between behavioral traits and task performance—task syndrome

Results of the PCA performed on the matrix of behavioral traits show the existence of two behavioral profiles: one group of workers displaying a bolder and more aggressive behavior and showing higher exploratory skills (higher total distance), and another group of workers characterized by less aggressive and bold behavior but also walking a more meandering pathway. Moreover, these behavioral profiles were linked to the performance of particular tasks by the workers. Bolder and more aggressive workers performed more *allogrooming* ($z = 2.03$, $p = 0.042$), less *brood care* ($z = -2.30$, $p = 0.021$), and also being *inactive* in the nest ($z = 2.30$, $p = 0.021$). On the contrary, less bold and aggressive workers were mainly focused on carrying out more *brood care* ($z = 2.63$, $p = 0.008$) and more *selfgrooming* ($z = -2.35$, $p = 0.019$). No other significant relationships were found.

Effect of behavioral traits on location switching

The switching workers that stayed for a longer time inside the nest (longer nurse stage) and those that never started to forage (not-switching workers) were less aggressive

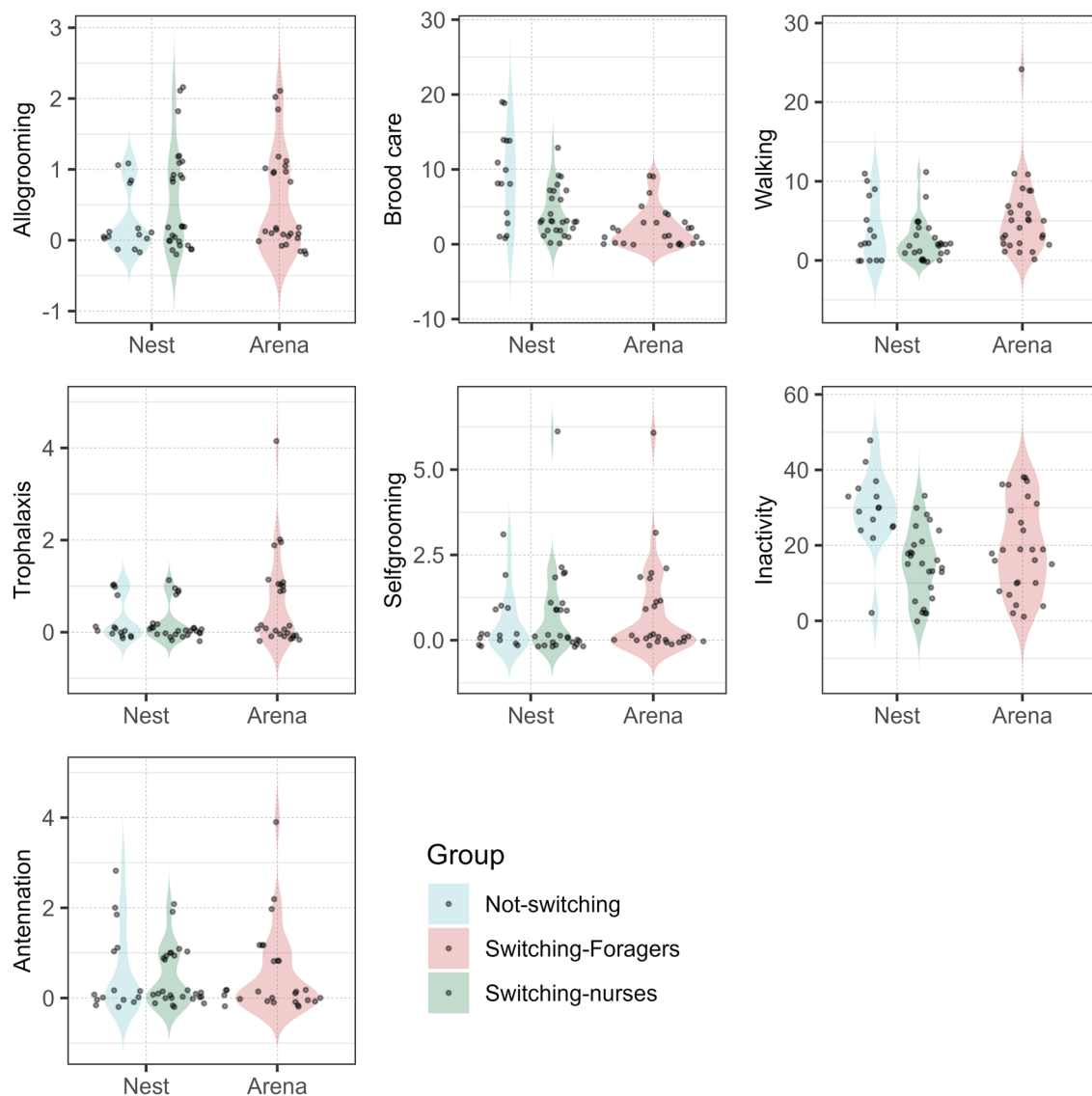


Fig. 2 Violin plots on the number of times each task was performed by the two casts of workers (not-switching and switching), before (Nest) and after (Arena) location switching

(*aggressiveness*: $z = -3.89$, $p < 0.001$), and walked shorter distances (*total distance*: $z = -5.81$, $p < 0.001$), and were less bold (*boldness*: $z = 5.0$, $p < 0.001$). Finally, the meanderiness of their path of movement (*total meandering*: $z = 0.178$, $p = 0.858$) was not related to the *location switching* of workers.

Effect of the lifespan and worker size on task performance and behavioral traits

Workers' lifespan varied from 51 to 143 days maximum. When testing if the individuals' lifespan influences the

performance of the different tasks, workers living longer performed less *antennation* ($z = -3.11$, $p = 0.002$) and spent less time *inactive* ($z = -2.83$, $p = 0.005$); but no relationship was found with the other traits (Table 1). Moreover, lifespan was shown to correlate with the *location switching* of the workers, as switching workers with longer lifespan performed *location switching* earlier in their life ($t = -3.09$, $p = 0.004$, Fig. 3).

The head width varied among workers showing a minimum value of 0.09 mm and a maximum value of 0.25 mm (standard deviation = 0.039) which indicates a difference in

Table 1 Relationship between worker's lifespan (n days) and worker size and the tasks performed by the individuals and their behavioral traits. In the table, it has been considered the tasks performed by the workers (see Table S1 for definition) and the workers' behavioral traits (*Aggressiveness* - scores obtained during the aggression assay; *Boldness* - time spent by the ant to leave the falcon tube and start foraging; *Total distance* - centimeters walked by the focal ant during the observation; and *Meandering* - changes in the direction of the focal ant's path measured in deg/cm).

vs Lifespan (n days)					
Performed Tasks	z	p	Behavioral traits	z	p
<i>Allogrooming</i>	-0.24	0.811	<i>Aggressiveness</i>	1.08	0.286
<i>Brood care</i>	-1.15	0.251	<i>Boldness</i>	-0.131	0.897
<i>Trophalaxis</i>	-1.33	0.183	<i>Total distance</i>	0.165	0.869
<i>Antenning</i>	-3.11	0.002	<i>Meandering</i>	0.49	0.621
<i>Walking</i>	-0.93	0.353			
<i>Selfgrooming</i>	-0.87	0.383			
<i>Inactivity</i>	-2.84	0.005			
vs Worker size (head width—mm)					
<i>Allogrooming</i>	1.29	0.196	<i>Aggressiveness</i>	3.06	0.004*
<i>Brood care</i>	0.22	0.828	<i>Boldness</i>	-0.03	0.978
<i>Trophalaxis</i>	-0.81	0.421	<i>Total distance</i>	-0.41	0.680
<i>Antenning</i>	-1.59	0.111	<i>Meandering</i>	2.11	0.041*
<i>Walk</i>	-2.31	0.021*			
<i>Selfgrooming</i>	-0.86	0.389			
<i>Inactivity</i>	-3.55	>0.001*			

the size of the studied workers. The head width was found to be correlated with the performed tasks such as *walk* and *inactivity* inside the nest, and with the behavioral trait—*aggressiveness*. Ants with larger size were seen walking in a lower number of observations (*walk*: $z = -2.31$, $p = 0.021$),

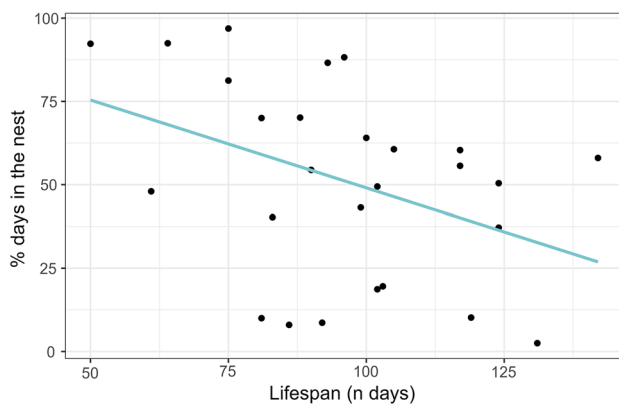


Fig. 3 Linear regression model between the time spent by the workers within the nest before *location switching* occurs and their lifespan. Both variables are measured as the number of days passed from the placing of the workers in the artificial nest till the *location switching* happened

were less inactive (*inactivity*: $z = -3.55$, $p < 0.001$), but were more aggressive (*aggressiveness*: $z = 3.06$, $p = 0.004$) and had a more meandering pathway ($z = 2.11$, $p = 0.041$). The size of the workers was not correlated either with their lifespan ($z = -1.47$, $p = 0.148$) nor with the time spent till the *location switching* ($z = -0.57$, $p = 0.567$). Finally, none of the other tasks or behavioral traits were found to be correlated with this morphometric variable (Table 1).

Repeatability of the behavioral traits

The assessed workers showed no significant consistency over time regarding *aggression* (RICC = 0, $N = 42$, CI95% [0, 0.143], $p = 0.5$) and *boldness* (RICC = 0.74, $N = 42$, CI95% [0, 0.253], $p = 1$), whereas *total distance* (RICC = 0.34, $N = 39$, CI95% [0.04, 0.59], $p = 0.01$) and *total meandering* (RICC = 0.29, $N = 39$, CI95% [0, 0.56], $p = 0.03$) were repeatable across two sessions.

Discussion

Our study showed that differences in the individual life history traits of the ant workers can be one of the factors influencing task performance and *location switching* and in turn, affect the division of labor. These results are in line with the study of Loftus et al. (2021), where the concept of task syndrome was described. We also show that *location switching* is not exclusively age-dependent since workers of the same age can remain (not-switching workers) and perform tasks only inside the nest, whereas others (switching workers) start to forage at an early stage of their lives. Specific behavioral traits were also linked with the performance of particular tasks in the colony. For example, workers who covered larger distances during the assays were those carrying out more allogrooming or walking within the nest, whereas workers with more meandering pathway were those taking care of the brood and walking less within the nest. In our study, the time of *location switching* was also associated with the behavioral traits of workers. Individuals who never switched locations or did it later in their life covered less distances in locomotory tests were less aggressive compared to those ants that started foraging earlier in their lives. Moreover, we found that switching workers tended to perform less *brood care* as they became older and, generally, they performed less *brood care* than not-switching workers. Finally, we also observed that worker size can also influence behavioral traits and task performance. Division of labor in ant colonies is linked to general characteristics of the individuals like the

age of the workers (age polyethism). However, even though all observed workers in our study were of the same age, they clearly differed in their task performance and in their tendency to leave the nest and start foraging. More than one-third of them performed only tasks inside the nest, whereas other workers showed *location switching* and were observed to forage already in the second week of their lives.

In our main hypothesis, we tested the existence of task syndrome (Loftus et al. 2021). In our study, the results of the tests showed a division of workers into two different behavioral profiles, which were characterized by behavioral traits and task performance. More aggressive and bolder workers covered longer *total distances* during the locomotory assays and carried out more *allogrooming* and less *brood care*, characteristics that constitute a very suitable profile for foraging. Notwithstanding, walking longer distances increases the risk of infection by pathogens, so carrying out allogrooming more frequently might reduce the colony's risk from pathogens infestations. Moreover, individuals moving more within the nest (*walk*) were less *bold*, moved straighter (lower *meandering*) and covered longer *total distances*; whereas those carrying out brood care moved in a more meandering pathway. In our study we observed workers walking within the nest on many occasions. This task named *walk* is similar to the one named *moving* in Cerdá and Retana work (1992), where this task was linked to not-switching workers and transporters. These groups of workers carried out activities of maintenance of the colony as well as transportation of the brood, two activities that required more efficient displacement. In *C. vagus*, however, these walking skills might be excellent allies for moving the nest in environmental disturbances or threats. As mentioned above, *brood care* is linked to a particular locomotory trait of the workers: the workers performing this task had a more *meandering* pathway during the movement. This meandering movement might be a useful skill for not-switching workers since such pathway characteristics offer higher chances of responding to sensory cues available in their surroundings (Buehlmann et al. 2012). For example, when a disturbance occurs in the nest, it could facilitate scattering the broods in a shorter time over a larger area. Altogether, these results support the existence of task syndromes through the changes in the behavioral profile of the workers according to the tasks performed in their life. It may suggest that the task specialization of workers can already appear at an early age under the influence of some specific traits, among which locomotory skills seem to play an important role. However, the combination of tasks and behaviors involved in task syndrome might vary among species as a result of their different life history traits.

In our study, we were also interested in testing whether similarity in the behavioral or locomotory traits of ants can determine the timing of location switching. As a

response to this question, we obtained the result that more aggressive workers switched earlier to foraging activities, whereas less aggressive workers stayed longer inside the nests. Moreover, as predicted, locomotory skills seem to significantly affect workers' task performance and location switching. The tardiest workers in location switching walked shorter distances during their locomotory tests. Ecologically, higher explorative skills during the forager stage might substantially increase the possibility of finding resources in larger amounts or with higher quality. Moreover, faster movement allows workers to reach more distant locations in a shorter time, which can lead to an increase in foraging efficiency. Workers switching locations earlier in their life were also more aggressive, a skill that can be important when defending the nest or monopolization of food sources. Similar to our study, a link between behavioral traits and task allocation has been found in different ant species. For example, *Leptothorax acervorum* worker ants that were less aggressive early in life performed more *brood care*. In addition, the workers that became more *aggressive* while ageing explored more than their same-age counterparts (Kühbandner et al. 2014). Furthermore, more docile individuals of *Temnothorax longispinosus* took care of the brood, while more aggressive individuals defended the nest (Modlmeier and Foitzik 2011; Modlmeier et al. 2012).

In this study, we hypothesized that the task performance in the colony is strongly linked to behavioral traits of workers in each existent caste. Similarly to the findings of previous studies (Cerdá and Retana 1992, Mersch et al. 2013), we found the existence of two castes showing differences in their task performance even before the *location switching*. For example, *brood care* and *selfgrooming* were mainly observed in not-switching workers, whereas switching workers explored a larger area of the nest by being often seen walking.

In our study, 64.3% of the workers showed *location switching* (from tasks performed inside the nests to those performed outside the nests), however, it happened at different age periods. Some of them started foraging within the first 20% of their life, whereas the rest of switching workers left the nest after 40% of their lifespan had passed. Therefore, workers' *location switching* seems to occur independently of their age and it could be influenced by individual differences in maturation (*ageing syndrome*; Jandt et al. 2014, Richardson et al. 2021).

We also hypothesize the existence of a relationship between the worker size and lifespan with their task performance and behavioral traits. Some authors have linked task allocation with worker size (caste polyethism) (Iwasa and Yamaguchi 2020; West and Purcell 2020) and suggest that larger workers can be in charge of external activities, whereas smaller workers usually stay inside the colony and medium-sized workers can remain more generalist, being

able to perform every type of task (Waddington et al. 2010). Our results are in agreement with these studies since they also show that larger workers were more *aggressive*, *walked* less but remained less time *inactive*; which describes a suitable profile to face the threats of carrying out activities like defense of the nest or trail foraging. Another individual variable connected with the location switching was the lifespan, and contrary to our expectations, workers spending shorter time carrying out tasks within the nest were those workers who lived longer. The same results were found by Kwapich and Tschinkel (2016), whose study showed how the increase in the longevity of *Pogonomyrmex badius* foragers benefits colony growth by decreasing the transition of new workers into foragers.

In our third hypothesis, we expected the repeatability of the behavioral traits of the workers across time. Contrary to our expectations, we did not find repeatability in *aggressiveness* and *boldness*. These changes in behavioral traits could be related to the behavioral plasticity of workers (Wright et al. 2019). Thus, as workers age, they express the most appropriate behavioral profile that allows for more efficient task performance (Richardson et al. 2021). On the other hand, we found that traits connected with worker movement like total distance and the meandering pathway were consistent over time. Such results together with the findings by Kühbandner et al. (2014), might highlight the importance of worker foraging skills as providing food incurs a fitness cost to the colony (Charbonneau et al. 2017).

In conclusion, we can point out that the studied behavioral traits are involved in the task allocation and performance of workers within a colony. Some behavioral differences between switching and not-switching workers are already present at an early age, although behavioral consistency can be stronger when the age of the workers is more advanced. Additionally, our results also underpin the previously found relationship between workers' behavioral traits and their foraging activity, which enhances the task allocation and performance of workers fulfilling such tasks. Therefore, our study supports the existence of task syndrome in ants. Such differences in the behavioral traits among colony members can lead to the higher efficiency of workers in the tasks they undertake and, in turn, can also increase the fitness of a colony.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00040-023-00937-0>.

Author contributions Conceptualization and study design: all authors; conceptualization of the idea and study design: MW; Laboratory work: GT, IEM, PS and MW; Analyses: GT and IEM; Writing: original draft preparation: GT; Writing: review and editing: GT, IEM, PS and MW. The final manuscript was approved by all authors.

Funding The study was carried out with the support of the Polish National Science Centre (2015/17/B/NZ8/02492).

Data availability All data used in this paper are available in a summary table as supplementary material Table S1. Additionally, the original data files are available in figshare scientific repository at <https://doi.org/10.6084/m9.figshare.22439200>.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Bates D, Maechler M, Bolker B, Walker S (2013) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0–5. <http://CRAN.R-project.org/package=lme4>
- Beekman M, Jordan LA (2017) Does the field of animal personality provide any new insights for behavioral ecology? *Behav Ecol* 28(3):617–623
- Bell AM (2007) Future directions in behavioural syndromes research. *Proceed Royal Soc B* 274:755–761
- Bernadou A, Busch J, Heinze J (2015) Diversity in identity: behavioral flexibility, dominance, and age polyethism in a clonal ant. *Behav Ecol Sociobiol* 69:1365–1375
- Bernadou A, Hoffacker E, Pable J, Heinze J (2020) Lipid content influences division of labour in a clonal ant. *J Exp Biol* 223:jeb219238
- Beshers NS, Fewell HJ (2001) Models of division of labor in social insects. *Annl Rev Entomol* 46:413–440
- Blight O, Villalta I, Cerdá X, Boulay R (2016) Personality traits are associated with colony productivity in the gypsy ant *Aphaenogaster senilis*. *Behav Ecol Sociobiol* 63(4):551–562
- Buehlmann C, Hansson BS, Knaden M (2012) Path integration controls nest-plume following in desert ants. *Curr Biol* 22(7):645–649
- Charbonneau D, Sasaki T, Dornhaus A (2017) Who needs 'lazy' workers? Inactive workers act as a 'reserve' labor force replacing active workers, but inactive workers are not replaced when they are removed. *PLoS ONE* 12(9):e0184074
- Carere C, Audebrand C, Rödel HG, d'Ettorre P (2018) Individual behavioural type and group performance in *Formica fusca* ants. *Behav Proc* 157:402–407
- Cerdá X, Retana J (1992) A behavioural study of transporter workers in *Cataglyphis iberica* ant colonies (Hymenoptera Formicidae). *Ethol Ecol Evol* 4:359–374

- Choe DH, Millar JG, Rust MK (2009) Chemical signals associated with life inhibit necrophoresis in Argentine ants. *Proc Natl Acad Sci USA* 109:8251–8255
- Corona M, Libbrecht R, Wurm Y, Riba-Grognuz O, Studer RA, Keller L (2013) Vitellogenin underwent subfunctionalization to acquire caste and behavioral specific expression in the harvester ant *Pogonomyrmex barbatus*. *PLOS Genet* 9:e1003730
- Cronin AL (2015) Individual and group personalities characterise consensus decision-making in an ant. *Ethology* 121:703–713
- Giehr J, Heinze J, Schrempf A (2017) Group demography affects ant colony performance and individual speed of queen and worker ageing. *BMC Ecol Evolut* 17(1):173
- Gordon DM (2013) The rewards of restraint in the collective regulation of foraging by harvester ant colonies. *Nature* 498:91–93
- Gordon DM (2016) From division of labor to the collective behavior of social insects. *Behav Ecol Sociobiol* 70:1101–1108
- Gordon DM (2019) The ecology of collective behavior in ants. *Annu Rev Entomol* 64:35–50
- Hartig F, Lohse L (2020) DHARMA: Residual diagnostics for hierarchical (multi-level / mixed) regression models. <https://CRAN.R-project.org/package=DHARMA>
- Hölldobler B, Wilson EO (1999) The ants. Harvard University Press, Cambridge
- Huxley JS (1912) The Individual in the Animal Kingdom. Cambridge University Press, London
- Iwasa Y, Yamaguchi S (2020) Task allocation in a cooperative society: specialized castes or age-dependent switching among ant workers. *Sci Rep* 10:3339
- Jandt M, Bengston S, Pinter-Wollman N, Pruitt JN, Raine NE, Dornhaus A, Sih A (2014) Behavioural syndromes and social insects: personality at multiple levels. *Biol Rev* 89:48–67
- Jeanson R, Weidenmüller A (2014) Interindividual variability in social insects-proximate causes and ultimate consequences. *Biol Rev* 89:671–687
- Kassambara A, Mundt F (2016) Factoextra: extract and visualize the results of multivariate data analyses. <https://CRAN.R-project.org/package=factoextra>, R package version 1.0.3
- Kühbandner S, Modlmeier A, Foitzik S (2014) Age and ovarian development are related to worker personality and task allocation in the ant *Leptothorax acervorum*. *Curr Zool* 60(3):392–400
- Kwapich CL, Tschinkel WR (2016) Limited flexibility and unusual longevity shape forager allocation in the Florida harvester ant (*Pogonomyrmex badius*). *Behav Ecol Sociobiol* 70:221–235
- Lê S, Josse J, Husson F (2008) FactoMineR: an R package for multivariate analysis. *J Stat Softw* 25(1):1–18
- Lenoir A, Aron S, Cerdá X, Hefetz A (2009) Cataglyphis desert ants: a good model for evolutionary biology in Darwin's anniversary year. A review. *Israel J Entomol* 39:1–32
- Lessells CM, Boag PT (1987) Unrepeatable repeatabilities: a common mistake. *Auk* 104:116–121
- Loftus JC, Perez AA, Sih A (2021) Task syndromes: linking personality and task allocation in social animal groups. *Behav Ecol* 32(1):1–17
- Maák I, Roelandt G, d'Ettorre P (2020) A small number of workers with specific personality traits perform tool use in ants. *Elife* 9:e61298
- Maák I, Trigos-Peral G, Ślipiński P, Grześ IM, Witek M (2021) Ant behavioural variation on individual and colony level and its effect on brood production depend on the habitat and colony characteristics. *Behav Ecol* 32(1):124–137
- Mersch DP, Crespi A, Keller L (2013) tracking individuals shows spatial fidelity is a key regulator of ant social organization. *Science* 340(6136):1090–1093
- Modlmeier AP, Foitzik S (2011) Productivity increases with variation in aggression among group members in *Temnothorax* ants. *Behav Ecol* 22:1026–1032
- Modlmeier AP, Liebmann JE, Foitzik S (2012) Diverse societies are more productive: a lesson from ants. *Proc Royal Soc B Biol Sci* 279:2142–2150
- Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol Rev Camb Philos Soc* 85:935–956
- Moroń D, Witek M, Woyciechowski M (2008) Division of labour among workers with different life expectancy in the ant *Myrmica scabrinoidis*. *Anim Behav* 75:345–350
- Norman VC, Hughes WOH (2016) Behavioural effects of juvenile hormone and their influence on division of labour in leaf-cutting ant societies. *J Exp Biol* 219(1):8–11
- Oksanen J, Guillaume Blanchet F, Friendly M, Kindt R, Legendre P, McGinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2019) Vegan: community ecology package. R package version 2.5–6. <https://CRAN.R-project.org/package=vegan>
- Pamminger T, Foitzik S, Kaufmann KC, Schützler N, Menzel F (2014) Worker personality and its association with spatially structured division of labor. *PLoS One* 9(1):79616
- Ravary F, Lecoutey E, Kaminski G, Chaline N, Jaisson P (2007) Individual experience alone can generate lasting division of labor in ants. *Curr Biol* 17:1308–1312
- R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>
- Richardson TO, Kay T, Braunschweig R et al (2021) Ant behavioral maturation is mediated by a stochastic transition between two fundamental states. *Curr Biol* 31(10):2253–2260.e3
- Sarkar D (2008) Lattice: multivariate data visualization with R. Springer, New York. ISBN 978-0-387-75968-5. <http://lmdvr.r-forge.r-project.org>
- Sih A, Bell A, Johnson JC (2004) Behavioral syndromes: an ecological and evolutionary overview. *Trends Ecol Evol* 19:372–378
- Stoffel MA, Nakagawa S, Schielzeth H (2017) rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods Ecol Evol* 8:1639–1644
- Tripet F, Nonacs P (2004) Foraging for work and age-based polyethism: the roles of age and previous experience on task choice in ants. *Ethology* 110:863–877. <https://doi.org/10.1111/j.1439-0310.2004.01023.x>
- Waddington SJ, Hughes WOH (2010) Waste management in the leaf-cutting ant *Acromyrmex echinator*: The role of worker size, age and plasticity. *Behav Ecol Sociobiol* 64(8):1219–1228
- West M, Purcell J (2020) Size-based task partitioning in ants lacking discrete morphological worker subcastes. *Behav Ecol Sociobiol* 74: 66
- Wickham H (2007) Reshaping Data with the reshape Package. *J Stat Softw* 21(12):1–20.
- Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York
- Wickham H (2011) The split-apply-combine strategy for data analysis. *J Statist Software* 40(1):1–29. <http://www.jstatsoft.org/v40/i01/>
- Wickham H, Seidel D (2022) scales: Scale Functions for Visualization. R package version 1.2.1. <https://CRAN.R-project.org/package=scales>
- Woyciechowski M, Kozłowski J (1998) Division of labor by division of risk according to worker life expectancy in the honeybee (*Apis mellifera* L.). *Apidologie* 29:191–205. <https://doi.org/10.1051/apido:19980111>
- Wright CM, Lichtenstein JLL, Doering GN, Pretorius J, Meunier J, Pruitt JN (2019) Collective personalities: present knowledge and new frontiers. *Behav Ecol Sociobiol* 73:31