Title: Flexible task allocation and raid organization in the termite-hunting ant *Megaponera analis*

Short Title: Raid organization in the ant *Megaponera analis*

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Author contributions

E.T.F. and K.E.L. designed the study. E.T.F. collected and analyzed the data and wrote the paper. Both authors discussed the results and commented on the manuscript.

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ABSTRACT

Division of labor is one of the main reasons for the success of social insects. Worker polymorphism, age polyethism and work division in more primitive ants, like the ponerines, remain mostly unexplored. The group-hunting, termite-specialist *Megaponera analis* conducts raids in column-formations of 200–500 ants. Since these ants specialize on a defensive prey, adaptations to reduce their foraging costs can be expected. We found that the work division, task allocation and column-formation during the hunt were much more sophisticated than was previously thought. The column-formation was remarkably stable, with the same ants resuming similar positions in subsequent raids and front ants even returning to their positions if displaced in the same raid, suggesting yet unknown regulatory mechanisms for the formation of the column. We identified three previously undescribed tasks during the hunting process of *M. analis*: lingerers, runners and raid-guards. Most of these tasks were not executed by predetermined members of the raid but were filled out as need arose during the hunt, with a clear preference for larger ants to conduct most tasks. The plasticity of task allocation was particularly well exemplified by the termite carriers, with the number of small ants carrying termites only starting to rise when less large ants were available. We therefore propose that the continuous allometric size polymorphism in *M. analis* allows for greater flexibility in task allocation, necessary due to the unpredictability of task requirements in an irregular system such as hunting termites in groups.

INTRODUCTION

Division of labor is a key characteristic in social insects, with the most obvious example being the queen focusing on reproduction while the workers focus on nest tasks and foraging (Hölldobler and Wilson 1990). Worker division of labor can arise from combinations of worker age, morphology, frequency distribution and dominance interactions (Hölldobler and Wilson 2008). Within behavioral castes, like brood care or foraging, further task partitioning can occur (like brood care focused on grooming or feeding). This behavioral caste membership can have a physiological or developmental basis (Hölldobler and Wilson 2008). There is a variety of evidence and debate about how fluid movement between behavioral castes is (i.e. foragers returning to brood care work) (Korczyńska et al. 2014, Herb et al. 2012), but it is well established that the partitioning of tasks during foraging, nest maintenance or brood care have a higher degree of flexibility (i.e. brood care workers may groom, feed or move larvae depending on current demands but are not recruitable for foraging) (Robinson et al. 2009). Work division in monomorphic species is generally believed to be regulated on the basis of age polyethism, with younger workers conducting nest tasks and later on performing tasks outside the nest, like foraging (Hölldobler and Wilson 2008). In addition to age polyethism, worker division of labor may also have a morphological basis (Hölldobler and Wilson 2008). Prominent examples of worker dimorphism are the soldier and worker caste, present in many ant genera, like *Pheidole* (Wilson 1984). There are also various examples of work division in polymorphic genera like *Atta* (Wilson 1980). These species generally partition their work by size, with brood and materials scaling with the size of the worker, i.e. smaller workers handle smaller larvae and food (Wilson 1980). In more primitive ants, like the ponerines, research on division of labor was mostly focused on reproduction, which is often regulated through policing and dominance hierarchies (Liebig et al. 1999). Worker polymorphism, age polyethism and work division in relation to foraging remain mostly unexplored though (Villet 1990). We hypothesize that group-hunting predatory species should show a large flexibility in their task allocations during foraging, since requirements for work division should be difficult to predict prior to the hunt (like number of caught prey, number of termite soldiers encountered). We therefore analyzed the raiding behavior of the ponerine ant species *Megaponera analis* and found that the work division, tasks and column-formation were much more sophisticated than was previously thought (Longhurst and Howse 1979; Bayliss and Fielding 2002).

Megaponera analis has caught the attention of various researchers for its very pronounced continuous allometric size polymorphism (Crewe et al. 1984; Villet 1990), a rare phenomenon in ponerines, and its specialization on group-hunting only termites of the subfamily Macrotermitinae (Longhurst et al. 1978; Yusuf et al. 2014).

Worker size in *M. analis* varies greatly, with majors being twice the size than minors (Schmidt and Shattuck 2014). *Megaponera analis* has a clear work division inside the nest, by partitioning their work by size, with the smaller workers taking care of the smaller larvae and eggs (Villet 1990). This work division is not only restricted to size but a clear age polyethism can also be observed, with younger workers conducting nest work and older workers going out to forage and scout (Villet 1990). This division of labor is not only confined to nest tasks but also plays a vital role in their rather unique foraging activity. The general foraging pattern of *M. analis* starts with scout ants searching an area of approximately 50 m radius around the nest for termite foraging sites (Frank and Linsenmair 2017; Bayliss and Fielding 2002). These scouts always belong to the largest ants in the colony (Longhurst and Howse 1979). Once a scout ant has found a potential hunting site it starts to investigate it, while avoiding contact with the termites, before returning in a direct route to recruit approximately 200–500 nestmates and lead them to the termites in a column like march formation (Bayliss and Fielding 2002; Longhurst and Howse 1979). The recruiting scout now is the raidleader and positioned at the front of the column followed by other large ants and scouts. The number of ants recruited by the scout depends on the number of termites at the hunting ground, in-line with what optimal foraging theory predicts (Frank and Linsenmair 2017). During the raid division of labor occurs (Corbara and Dejean 2000); larger ants break open the protective soil cover created by the termites while the smaller ants rush into these openings to kill and pull out the prey (Corbara and Dejean 2000). After the hunt the larger ants collect the dead termites, the column forms again and the hunting party returns together to the nest. More recent studies even show the existence of rescue behavior, with ants that got injured during the raid in the form of lost extremities and clinging termites getting carried back to the nest to recover (Frank et al. 2017).

This rather unique group-raiding behavior among ants made us wonder how task allocation worked when the necessity of some tasks could only be determined after the hunt (due to the unpredictability of termite yield at the foraging sites). While size polymorphism seems to play an important role in task allocation (Corbara and Dejean 2000), we do not know how the tasks after the hunt are distributed. The larger ants are generally the termite carriers, but we often observe the number of killed termites to exceed the number of available large ants. We therefore analyzed and filmed raids in the savannah of the Comoé National park to better understand how work division is allocated after the hunt, how the differently sized ants distribute themselves within the column (formation) and how many tasks are conducted during the hunting process.

Our study revealed much more specializations during the raid than previously thought (Longhurst and Howse 1979; Villet 1990), with newly defined behaviors, a highly sophisticated formation within the column and large flexibility in task allocation.

METHODS

Study area and organism

The study area is a humid savannah woodland located in the Comoé National Park, northern Côte d'Ivoire (Ivory Coast), at the Comoé National Park Research Station (8°46'N, 3°47'W). The annual rainfall is 1500-2200 mm, mostly falling from May to September (Konaté and Kampmann 2010). The termite hunting ant species *M. analis* was observed in a total of 54 different colonies for a total of 450 raids that predominantly hunted termites of the genus *Pseudocanthotermes* sp.. All colonies were located in a radius of approximately 2000 m from the research station and the distances between them varied between 10 to 200 m. Nests were most commonly located by following a raiding column or scout ant return to the colony. Colony size for 12 excavated colonies was between 900-2300 ants, a result comparable to previous studies in other regions (Villet 1990; Yusuf et al. 2013).

Data collection

Observations throughout the day in April 2013 established that raiding activity was highest in the morning and afternoon hours between 6:00-11:00 and 15:00-19:00 local time, which corresponds to prior observations (Bayliss and Fielding 2002; Longhurst and Howse 1979; Yusuf et al. 2014). Night raiding was also observed, but was not included in this study. Experiments and observations were therefore carried out in the field from 7:00-11:00 and 15:00-18:00 from April to September 2013, August to October 2014, January to March and July to September 2015 and March to May 2016. Even though *M. analis* is known to show monophasic allometry within its worker sizes (Crewe et al. 1984; Villet 1990), for statistical analysis and illustration, the workers were divided into larger ants (head width > than 2.00 mm) and smaller ants (head width < 1.99 mm), an intermediate cast (head width 2.40 - 1.99 mm) as proposed by Villet (1990) was not quantified in this study, since exact measurements were not possible without disturbance.

Raid composition

Data for the raid composition was collected by filming the entire raiding column on its outward and return journey and then watching the film in slow motion, assigning every single ant in one of six different categories: large or small ant, large or small ant carrying termites, large or small ant carrying injured nest mates. The position of every single ant in the column was also noted. This was done in a total of 14 raids, distributed as evenly as possible between six different colonies (*N*: colony A= 3, colony B= 1; colony C= 1, colony D= 4, colony E= 3, colony F= 2). To see how the composition of the different categories changed within the raiding column the column was divided into 10 equally large blocks each comprising 10% of the total number of ants participating in the raid. Since the raid size varied heavily within the sample size (possibly skewing the data within the blocks) we also examined the first and last 20 ants of the raid column.

Work division

In the first three raids of a colony (in a total of three colonies) all ants carrying out a certain task were marked (marking time was between 1-2 days). Ants were marked with acrylic two-color code on the thorax (four colors used: red, blue, green, gold) depending on their position (front, center, tail) and task. We differentiated the following tasks: scout, runner, helper, termite carrier, raid-guard, lingerer, termite hunter (definition of tasks is given in the results section). In the subsequent two weeks all raids carried out by the colonies were observed and the roles and behaviors of the marked ants was quantified. The relation of large and small termite carriers was calculated by using an exponential fit: y=a*e^(b*x), with a=0.452 and b=0.057.

Sensitivity to alarm pheromones was tested by picking up a large ant from the raiding column (without causing distress in the raiding party) and holding it with forceps 20cm away from the front of the returning raid column (until the whole column moved past the point). Ants attracted towards the forceps and biting it were quantified into three categories: large ant, small ant, termite carrier (always large); helpers carrying injured ants were never observed to be attracted.

Position fidelity

Twenty large ants were marked for each category (front, center, tail) in three colonies and their positions were quantified in subsequent raids during the next two weeks. For position fidelity in the same raid the ants were picked up with forceps and placed at the opposite position of the column (front ants at the tail and vice versa; center ants either at front or tail). Afterwards the ants were observed until they resumed a normal column speed (no further change in position) and the new position was quantified.

Statistical analysis

We used the statistical software R v3.1.2 (R Core Team 2013) with the user interface RStudio v0.98.501 and the R package ggplot2 v2.1.0 (Wickham 2009) for statistical analysis and illustration. We tested for deviations from the normal distribution with the Shapiro Wilks test (P>0.05). A Bartlett test was used to verify homoscedasticity (P>0.05). If data were normally distributed and homoscedastic an ANOVA was used to compare the significance of the results and to test if colony differences were significant (which was never the case), a Tukey HSD test was used for post hoc analysis. If this was not the case a Kruskal-Wallis rank sum test was used, followed by a Dunn's test with Holm-Bonferroni correction. Box-and-whisker plot show median (horizontal line), interquartile range (box), distance from upper and lower quartiles times 1.5 interquartile range (whiskers), outliers (> 1.5X upper or lower quartile) and significant differences (different letters). Linear correlations were calculated with a Pearson's rank sum test. A Kolmogorov-Smirnov Test with Bonferroni correction was used to test for position fidelity of ants in subsequent raids, values were tested against a hypothetical random distribution of marked ants within the column. A Fisher's exact test with Bonferroni correction was used to test for position fidelity of displaced ants within the same raid, values were tested against a hypothetical group that would show no fidelity.

RESULTS

Raid column formation

During the outward journey a clear overrepresentation of large workers was present at the front and tail of the column (Fig. 1a and Online Resource 1). On the return journey large workers tended to be more present at the front half of the column, although a disproportionate amount of large ants was again present at the tail of the column (Fig. 1b and Online Resource 2).

Work division

We identified seven different roles during the foraging process of *M. analis* for the larger ants and three for the smaller ants (Table 1).

Scouts

Scout ants always belonged to the largest ants in the colony (*N*=100 scouts), although only a fraction of large ants were scouts at a time. Only 14±5 scouts were active per foraging activity period (*N*=10) in colony sizes between 700-2000 individuals. The tasks of a scout involved leaving the nest at the beginning of the activity period, searching for food sources, investigating them and recruiting nestmates to the food source. At this point the scout became the raidleader. During the hunt itself the raidleader did not participate in the hunting process, standing at the periphery of or moving around the hunting ground. After the hunt when the ants started to gather again the raidleader was observed to join the column with the last 10% of the returning ants (*N*=10 raids), but then clearly moved forward to be at the front of the column.

When the raidleader recruited ants to a foraging site there were always formerly marked scouts that also joined the raid. Former scouts are ants that normally scout for food sources but decided to participate in a raid instead (before resuming their normal scouting behavior in later activity periods). Former scouts also showed a clear overrepresentation at the front of the column during the outward journey (40% of marked scouts; *N*=15 raids;

Table 2). Once at the hunting ground these ants did not participate in the actual hunt but instead positioned themselves at the periphery of the hunting ground, potentially searching for nearby termite sites. Furthermore, the first ten ants initiating the return of the column back to the nest were in 20 ± 16 % of the cases front ants from the outward journey, while marked ants from the center contributed 4 ± 5% and tail ants were never observed to lead a return column (*N*=5 raids; definition: front: first 10%; center: 10-90%, tail: last 10% of the column).

Termite carrier

The second already known task was that of the termite carriers. After the hunt the killed termites were piled up on the foraging ground and the ants started to collect them in their mandibles, forming balls of up to 12 termite workers. In an average raid this task was carried out by the larger ants in the colony (Fig. 2), which generally only needed 30% of the ants to carry all killed termites (median termite carriers per raid 29 ± 19%; *N*=134 raids). However, if the raid was overly successful more and more smaller ants carried termites, resulting in a positive exponential relation between smaller and larger termite carriers (Pearson test of ln small carriers against large carriers: t_{12}=7.2; R^2=0.81; P<0.001; Fig. 2). If more than 63% of the larger ants had to carry termites a tipping point was reached. After this point the increase of smaller ants performing this task was greater than the increase for larger ants (i.e. the slope of the exponential curve was larger than one; Fig. 2).

Termite carriers on the return journey positioned themselves towards the front of the column, although not in the first 10% block of the column, which was occupied by non-carrying large ants (raid-guards; Fig. 3; Online Resource 3 and 5). This formation could only clearly be recognized in raids that were not oversaturated with prey (<70% termite carriers; Fig. 3a; Online Resource 3 and 4). In oversaturated raids (>70% termite carriers) no clear formation was recognizable for the termite carriers (Fig. 3b; Online Resource 5 and 6).

Helper

Injured ants were always carried by larger ants (*N*=154 helper ants). The injured ants were mostly picked up during the phase when the termites were collected at the hunting ground, but were also picked up during the return journey. A median of 3 ± 2.9 ants conducted this task per raid. Helpers showed no clear position fidelity in the column on the outward and return journey (Table 2).

Lingerer

The first newly described role in this study was the lingerers. These ants actively returned to the hunting ground for another 1-2 minutes (*N*=10 raids) after the returning raid column started to form and leave for the nest. A mean of 5 ± 1 ants per raid conducted this task (*N*=11 raids). These ants were always large ants that did not carry anything (*N*=51 lingerers). They ran over the foraging site and showed a clear intention to pick up left over live ants or in rare cases termites (Fig. 4). After picking up a termite or an ant they immediately tried to return to the column. While lingerers started at the tail of the returning raid column, they showed no clear position fidelity within the column afterwards (Table 2).

Runner

Runners were ants that during the returning raid column moved in the opposite direction of the moving raid at the sides of the column from the front to the tail and overshot the column by up to 1 meter. These ants were always large and did not carry anything (*N*=17 runners), with generally having a mean of 1.4 ± 0.5 ants conducting this task per raid (*N*=12 raids). These ants picked up fallen termites or injured ants if encountered, although these incidences were rare (17% picked up a termite; 23% picked up an ant; 59% remained empty; *N*=17 runners).

Raid-guard

In the returning raid column all large ants that did not perform one of the previously described roles were classified as raid-guards, i.e. non-carrying large ants in the column. When an alarm pheromone of *M. analis* was elicited in the vicinity of the column (20 cm distance) the raid-guards were always the first ones to respond and attacked the source of the distress, unlike small ants with empty mandibles or termite carriers, which did not seem to react to it (raid-guards= 77 ± 10 %; Small ants= 12 ± 10 %; Termite carriers 11 ± 7%; *N*= 12; ANOVA: F_2=176; P<0.001; Tukey HSD: guard vs small ants: t_{11}=-16; P<0.001; guard vs termite carrier: t_{11}=-16; P<0.001; small ant vs termite carrier: t_{11}=-0.38; P=0.92). The raid-guards showed, just like on the outward journey, a clear preference for a position at the front or tail of the column, irrespective of the saturation of termite carriers (Fig. 3). In addition to defense, raid-guards at the tail of the column seemed to act as a "safety net", picking up fallen termites or injured ants when offered (92% of offered termites picked up; 85% of injured nestmates; *N*=13).

Position fidelity

Ants were marked during the outward journey of a raid for their respective positions. These ants were observed for the next two weeks to see if they held position fidelity in subsequent raids. Front ants showed a clear fidelity to be at the front of the column on the outward journey (Table 3 and Online Resource 7). On the return journey these ants were at the front (Table 3 and Online Resource 7) but over time started to fall back, showing no clear position fidelity by the time the column was quantified (Table 3 and Online Resource 7). Center ants were always observed to be in the center of the column, both on the outward and return journey (Table 3 and Online Resource 7). Tail ants tended to stay at the tail but were also found in the center of the column in subsequent outward journeys. On the return journey tail ants were either at the front or center.

We displaced ants from their positions during the outward and return journey. Front ants were picked up and placed at the tail and vice versa; center ants were placed either at the front or tail. Front ants showed clear intention to return to their position on the outward journey, overtaking the entire column and returning to their original position within minutes (as shown in the video Online Resource 8, Table 4 and Online Resource 9). On the outward journey tail and center ants also seemed to return to their former position.. On the return journey no clear position fidelity could be observed (Table 4 and Online Resource 9), independent from the earlier position.

DISCUSSION

We were able to identify three new tasks during the hunting process of *M. analis*: lingerers, runners and raid-guards. Most of these tasks seemed not to be predetermined before the raid but were filled out as need arose during the raid and were mainly conducted by the larger ants. The formation within the raid column was also much more sophisticated than previously thought (Longhurst and Howse 1979), with larger ants being present both at the tail and front of the column and performing special tasks, like patrolling on the sides of the column (runners). Furthermore, the formation was more stable than expected, with the same individuals resuming similar positions in subsequent raids and front ants even returning to their position if experimentally displaced in the same raid.

Column formation

Column formation in previous studies of *M. analis* focused on the front of the column (Longhurst and Howse 1979). We can confirm the results from Nigeria that more former scouts and large ants were present at the front and that these ants seemed to initiate the return journey (Fig. 1) (Longhurst and Howse 1979). In addition we now showed that the formation is even more complex, with termite carriers concentrated at the center of the column and a rearguard predominantly occupied by individuals best adapted to fighting off potential predators (raid-guards, Fig. 3) (Breed and Harrison 1988; Dejean and Feneron 1996; Hölldobler and Wilson 1990).

All these positions are ecologically meaningful. The role of front ants during the return journey could be guiding the column back to the nest in case of trail disturbance. While it has been confirmed multiple times that *M. analis* follows a pheromone trail back to the nest (Hölldobler et al. 1994; Longhurst and Howse 1979), this trail could be interrupted by external influences. Former scouts, which are accustomed to moving around the environment without a pheromone trail, could thus be better suited to lead the way back to the nest in case the pheromone trail disappeared, which some qualitative observations of trail disturbance by us seemed to suggest (pers. obs.). The more vulnerable termite carriers are best protected at the center of the column, these ants would arguably have more difficulties following a pheromone trail or fending off predators when carrying up to 12 termites in their mandibles. A further benefit of the rear-guard, apart from fending off predators, comes in the form of picking up lost prey or injured individuals, which are essential in colonies of *M. analis* (Frank et al. 2017).

Raid-guards are already known for instance in the driver ant genus *Dorylus*, with guards standing still, facing outwards, with open mandibles for long periods of time on the sides of a column (Hölldobler and Wilson 1990). While this is a good strategy in a mass-raider with ant columns lasting for days, this strategy would be less efficient in a species in which the entire column passes a certain point in one to two minutes (like *M. analis*).

The sophisticated formation in *M. analis* shows adaptations to their rather unique foraging behavior, which most likely improve their defense capabilities. The underlying mechanisms regulating this formation (how the ants "know" where to position themselves) remain unclear though and are discussed in the next section.

Position fidelity

Ants that were displaced from their position during the journey to the termites showed a surprisingly strong fidelity to return to their approximate former position. This was especially the case for front ants on the outward journey.

This suggests that the ants directly behind the raidleader might have a special role in the foraging process. Considering that most of these ants are former scouts that have led raids before we hypothesize that they are more experienced in interpreting signals of the raidleader. For example, the raidleader stops approximately 20 cm in front of the raiding site, giving the ants in the column a chance to gather before attacking together (Bayliss and Fielding 2002). The front ants might be more sensitive in recognizing this stop signal and could amplify it for the rest of the column. The first 10-20 ants might also have the additional role of strengthening the pheromone trail laid by the raid leader, thereby facilitating trail following for the other 500 ants (with 3-5 standing beside each other in the column). On the return journey no position fidelity was observed by the front ants, due to lack of necessity (the pheromone trail already reinforced and no raidleader giving signals).

The position fidelity observed from tail ants during the outward journey might be an artifact. These ants were never observed to actively move back to the tail of the column (after experimental displacement) but seemed to be disoriented and not moving forward until the tail of the column had caught up with them. During the return journey no real position fidelity was observed.

The observations of position fidelity in some ants raise questions on the underlying mechanisms regulating this behavior: how do ants know their position in the column? One possibility could be the intensity of the pheromone trail. Since all ants in the column are observed to lay a pheromone trail on the outward journey the intensity of this trail decreases the further one moves to the front. Another possibility could be unique volatiles emitted by the raidleader letting the ants know they are at the front. This second hypothesis could be supported by the fact that removal of the raidleader during the outward journey leads to immediate searching behavior by the ants (Longhurst & Howse 1979), suggesting individual recognition. Both of these hypotheses do not allow for position recognition during the return journey (were the raidleader is no longer at the front, if at all present), which might explain why we do not observe position fidelity during the return.

Plasticity of task allocation

Most of the observed tasks were not predetermined but were filled out as need arose during the raid, with a clear preference for the larger ants to conduct most tasks. Smaller ants were usually restricted to hunting termites in their tight galleries but also showed a certain plasticity by acting as termite carriers when necessary (i.e. when less large ants were available) (Fig. 2). The regulation of termite carrier number and size could be a passive process. Large ants tend to wait outside the galleries for the raid to finish, since they are too big to enter them. They thus might start gathering termites earlier and by the time smaller ants come out of the galleries there is nothing left to carry, unless the raid is oversaturated.

Otherwise all observed tasks were carried out by large ants. This makes sense from a purely morphological perspective. Larger ants are better adapted for carrying termites or small ants (which make up over 90% of the ants that need help) (Frank et al. 2017), thus being better suited for the task of helpers, termite carriers, runners and lingerers, all roles which to some extend complement each other in finding termites and injured ants. The larger size allows them to move faster in the environment and overcoming obstacles, thereby covering more ground in less time (Kaspari and Weiser 1999), likely making them better suited as scouts. Larger ants also generally are better soldiers or guards, with stronger and larger mandibles (Breed and Harrison 1988; Dejean and Feneron 1996; Hölldobler and Wilson 1990).

The only task that seemed to be predetermined, i.e. always conducted by the same ants, was the scouting behavior. Scout ants were most likely the oldest ants in the colony; since foraging alone outside of the nest is one of the riskiest tasks in the colony and age polyethism related to dangerous tasks is well known in ants (Villet

1990; Hölldobler and Wilson 2008). These individuals also participated in other raids, if available, and behaved markedly different to other raid-members, being placed behind the raid leader and the periphery of the hunting ground (former scouts).

In an unpredictable system, which the group hunting of termites represents, a large plasticity in task allocation seems beneficial. The number of injured nestmates or prey cannot be accurately estimated beforehand. While the size of the raid varies depending on the quality of the foraging site (Frank and Linsenmar 2017), a large uncertainty still remains. The wide range of different predated termite genera (*Odontotermes*, *Pseudocanthotermes*, *Macrotermes* etc.) makes the outcome of a hunt even more difficult to predict (injury rate and number of termite carriers should vary depending on the genera/species and foraging site). We therefore believe that at the end of a raid a large proportion of ants first try to pick up termites and injured nestmates. If this is not possible they then switch to the other tasks (lingerers, runners or raid-guards) and change their response threshold to outside stimuli accordingly (i.e. raid-guards being more sensitive to alarm pheromones). Interestingly, even in oversaturated raids (Fig. 3b) there were always large ants that did not carry termites but instead conducted the other tasks, some further thresholds or underlying mechanisms might thus influence task allocation during a raid.

This necessary plasticity in task allocation could explain the large continuous allometric size polymorphism in *M. analis*, with the smallest ants being half the size of the largest (Villet 1990). Thus, while intermediately sized ants might not be best suited for a certain task, they allow for a greater flexibility in task allocation. We therefore hypothesize that size polymorphism in *M. analis* mainly arose due to their unique foraging specialization on termites of the subfamily Macrotermitinae, rather than to handle smaller larvae and eggs, as suggested by Villet (Villet 1990). This is also supported by studies on the polymorphic species *Neoponera laevigata* (Hölldobler and Traniello 1980) and *Centromyrmex bequaerti* (Dejean and Feneron 1996), both of which are polymorphic and specialized termite predators.

Mechanisms regulating task allocation

The mechanisms regulating division of labor and task allocation leading to collective behaviors are still being debated in social insects (Jeanne 2016; Naug 2016; Gordon 2016). Gordon argues that interactions among workers and their environment (distributed processing) suffice to explain the collective behaviors we observe in ants (Gordon 2016). We agree that in cases where a large flexibility in task allocation is necessary and decisions have to be made in small time windows this can be an important mechanism, in *M. analis* this seems to be the case after the hunt (who becomes a termite carrier, who looks for/helps injured ants, who becomes a raid-guard).

There are certain patterns though which in our opinion cannot be explained without considering other factors, like age-polyethism and morphology (ant size). Task partitioning in the co-operative load transport of *Messor barbarous* for instance seems to be regulated solely by ant size and very simple rules: larger ant takes food from smaller ant (Anderson et al. 2002; Reyes and Fernández Haeger 1999). All observed tasks in *M. analis* (except direct termite hunting) are first conducted by large ants and only if necessary by smaller ants. The response threshold to participate in these tasks therefore must be lower in larger ants compared to smaller ants to explain this discrepancy (Bonabeau et al. 1998). Furthermore, the observation that only large ants become scouts and remain scouts for subsequent days (i.e. persistent individual specialization), implies that age (experience?) might play a role in the division of labor. We agree with Jeanne (2016) that there is a clear benefit for division of labor in polymorphic species such as *M. analis*, otherwise the emergence of this polymorphism would not make sense in our opinion.

Conclusion

We were able to show a remarkable amount of tasks and formation patterns within the raiding behavior of *M. analis*. This raises various interesting questions on the underlying mechanisms regulating the formation, work division and task allocation. We propose that continuous allometric size polymorphism in *M. analis* evolved mainly to allow for greater flexibility in task allocation, necessary due to the unpredictability of task requirements in an irregular system such as group-hunting of termites. We therefore believe that the unique foraging behavior of *M. analis* offers itself as a good model to study different mechanisms and factors regulating task allocation and work division in ponerine ants.

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TABLES

Table 1 Different tasks carried out during the foraging process. X: Task always carried out by this size class. *: Task only carried out when necessary.

	Work tasks during foraging (principal categories)						
Size class	Scout	Termite carrier	Helper	Runner	Lingerer	Raid-guard	Termite hunter
Large ant	X	X	X	X	X	X	*
Small ant		*				*	X

Table 2 Position of ants in the raid column. With front being the first 10%, tail being the last 10% and center the rest of the column in between. X: over represented in this position. *: also present but less frequently in this position. n.s.: no clear position.

	Work tasks during foraging (principal categories)						
Column position	Scout	Termite carrier	Helper	Runner	Lingerer	Raid-guard	Termite hunter
Front	X		n.s.	NA	n.s.	X	
Center	*	X	n.s.	NA	n.s.	*	X
Tail			n.s.	NA	n.s.	X	

Table 3 Position fidelity of ants in the raid column over subsequent raids for outward and return journeys. With front being the first 10%, tail being the last 10% and center the rest of the column in between. Disproportionately large deviations from the random distribution (Front: 10%, Center: 80%, Tail 10%) for the different categories marked in bold (see Online Resource 7 for detailed statistical results). Standard deviation in brackets.

Column position	Percentage of marked ants at position					
	Outward Journey			Return Journey		
	Front	Center	Tail	Front	Center	Tail
Front	**46**	7	**0**	14	25	40
	(26-71)	(0-11)	**(0-4)**	(0-35)	(0-50)	(20-45)
Center	**43**	79	75	**61**	75	60
	(27-59)	(78-86)	(63-100)	**(43-69)**	(50-100)	(55-80)
Tail	**0**	11	17	15	**0**	**0**
	(0-16)	(0-20)	(0-29)	(0-30)	**(0-0)**	**(0-0)**

Table 4 Position fidelity of ants displaced from their position in the same raid column, both on the outward and return journey. With front being the first 10%, tail being the last 10% and center the rest of the column in between. Significant values marked in bold (see Online Resource 9 for detailed statistical results).

Position fidelity	Percentage of marked ants at position					
	Outward Journey			Return Journey		
	Front	Center	Tail	Front	Center	Tail
Fidelity	**90%**	**70%**	**80%**	0%	30%	10%

FIGURES

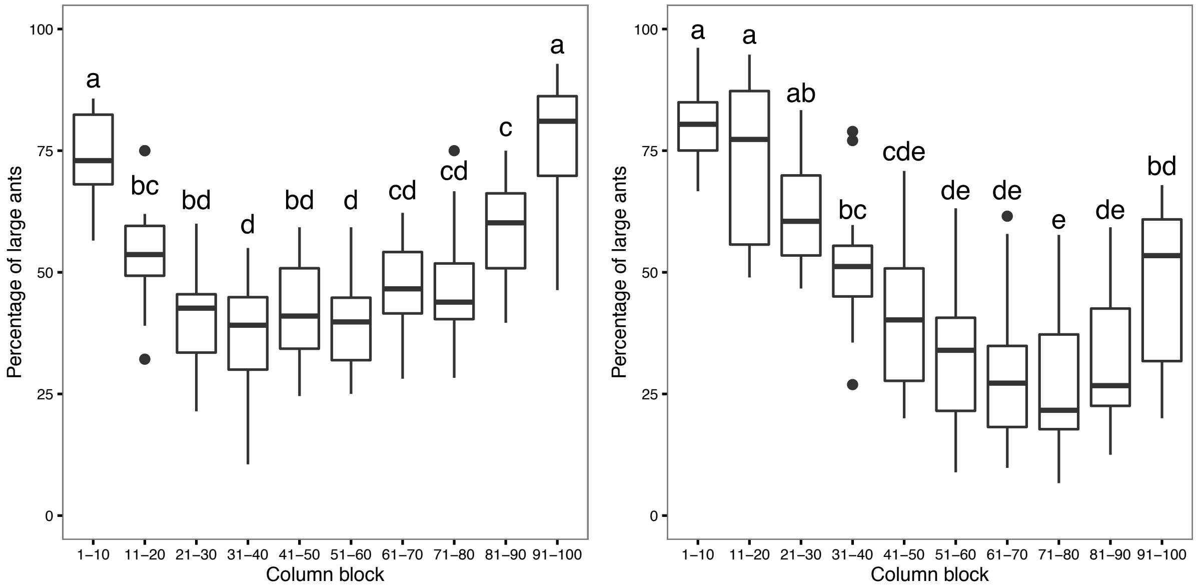

Fig. 1 Raid column formation. Representation of the larger and smaller workers of *M. analis* within the raiding column in 10% blocks of the column, with 1-10% being the front and 91-100% being the last 10% of the column (see Online Resource 1 and 2 for detailed statistical results). (a) Raid column on the way out to the termites. (b) Raid column on the return journey from the termites.

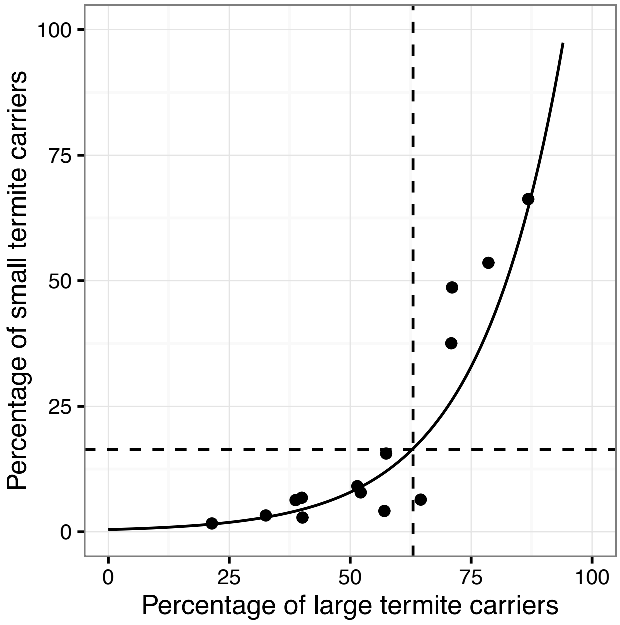

Fig. 2 Relation of large to small termite carriers. Plot of small termite carriers against large termite carriers quantified during the return journey for differently saturated raids. Black line: exponential fit (formula: $y=a*e^{b*x}$). Dashed line intersection point: point were more small ants start to conduct the task compared to large ants (slope=1).

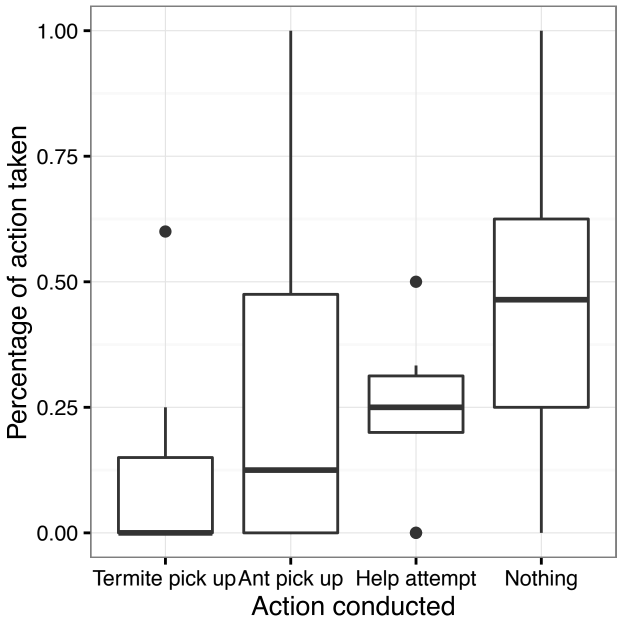

Fig. 3 Formation of termite carriers and raid-guards in the returning raid column. Representation of the larger and smaller workers of *M. analis* within the raiding column in 10% blocks of the column, with 1-10% being the front and 91-100% being the last 10% of the column (see Online Resource 3 – 6 for detailed statistical results). **(A)** Normally saturated raid (<70% termite carriers). **(B)** Oversaturated raid (>70% termite carriers).

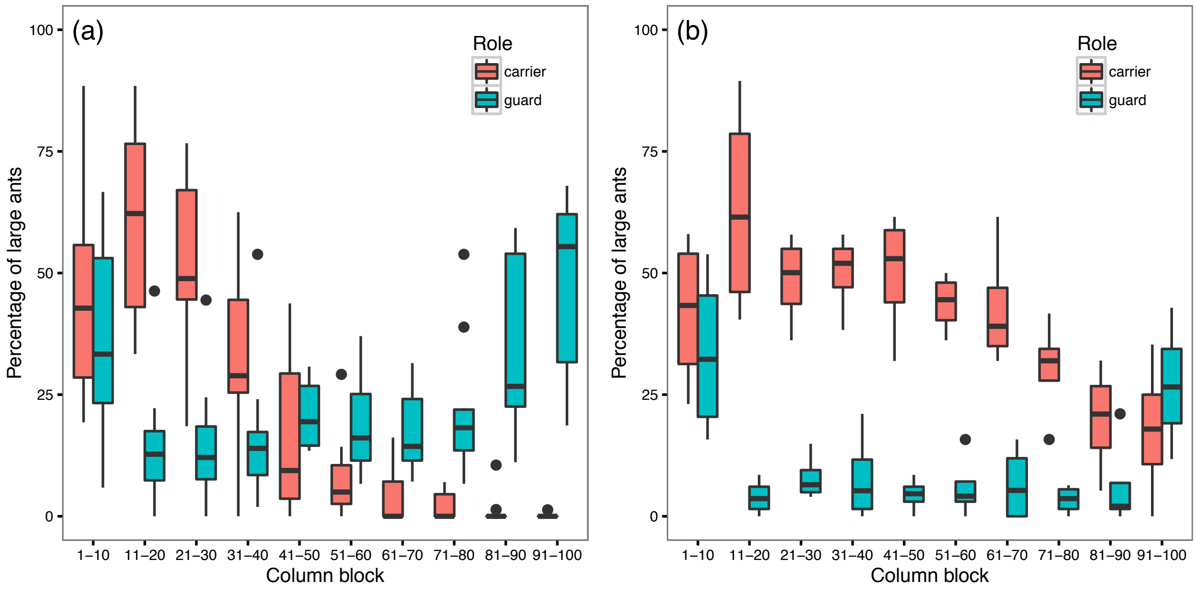

Fig. 4 Percentage of actions observed in lingerer ants. Termite pick up: picking up a dead termite. Ant pick up: picking up an ant still at the hunting ground. Help attempt: trying to pick up an uncooperative ant. Nothing: not encountering any of the previous categories and leaving empty.