Behavioural adaptations in the foraging behaviour of *Megaponera analis*

Verhaltensanpassungen im Furagierverhalten von *Megaponera analis*

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submitted by

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“[…] I observed many regiments of black soldier-ants, returning from their marauding expeditions… They are black, … about half an inch in length and on the line of march appear three or four abreast; when disturbed, they utter a distinct hissing or chirping sound. They follow a few leaders who never carry anything, and they seem to be guided by a scent left on the path by the leaders; … When on their way to attack the abode of the white ants, the latter may be observed rushing about in a state of great perturbation. The black leaders, distinguished from the rest by their greater size… then seize the white ants one by one, and inflict a sting, which… renders them insensible but not dead, and only able to move one or two front legs. As the leaders toss them on one side, the rank and file seize them and carry them off… these black ruffians are a grade lower than slave-stealers, being actually cannibals.”

[David Livingstone: Missionary Travels and Researches in South Africa, 1857]

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I hereby confirm that my thesis entitled Behavioural adaptations in the foraging behaviour of Megaponera analis is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

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Hiermit erkläre ich an Eides statt, die Dissertation Verhaltensanpassungen im Furagierverhalten von Megaponera analis eigenständig, d.h. insbesondere selbständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

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Megaponera analis ants breaking up the protective soil layer over a termite foraging site.

SUMMARY

An efficient foraging strategy is one of the most important traits for the fitness of animals. The theory of optimal foraging tries to predict foraging behaviour through the overarching question: how animals should forage so as to minimize costs while maximizing profits? Social insects, having occupied nearly every natural niche through widely different strategies, offer themselves as an ideal group to study how well optimal foraging theory can explain their behaviour and success.

Specialization often leads to unique adaptations in morphology and behaviour. I therefore decided to investigate the behaviour of *Megaponera analis*. This ponerine ant species is specialized on hunting only termites of the subfamily Macrotermitinae at their foraging sites. Their foraging behaviour is regulated by a handful of individual scouts (10-20) that search for termite foraging sites before returning to the nest to recruit a large number of nestmates (200-500 ants). These ants then follow the scout in a column formation to the termites and after the hunt return together to the nest, these raids occur two to five times per day.

Predators of highly defensive prey likely develop cost reducing adaptations. The evolutionary arms race between termites and ants led to various defensive mechanisms in termites, e.g. a caste specialized in fighting predators. As *M. analis* incurs high injury/mortality risks when preying on termites, some risk mitigating adaptations have evolved. I show that a unique rescue behaviour in *M. analis,* consisting of injured nestmates being carried back to the nest, reduces combat mortality. These injured ants “call for help” with pheromones present in their mandibular gland reservoirs. A model accounting for this rescue behaviour identifies the drivers favouring its evolution and estimates that rescuing allows for maintaining a 29% larger colony size. Heavily injured ants that lost too many legs during the fight on the other hand are not helped. Interestingly, this was regulated not by the helper but by the uncooperativeness of the injured ant. I further observed treatment of the injury by nestmates inside the nest through intense allogrooming directly at the wound. Lack of treatment increased mortality from 10% to 80% within 24 hours, with the cause of death most likely being infections.

Collective decision-making is one of the main mechanisms in social insects through which foraging is regulated. However, individual decision-making can also play an important role, depending on the type of foraging behaviour. In *M. analis* only a handful of individuals (the scouts) hold all the valuable information about foraging sites. I therefore looked at predictions made by optimal foraging theory to better understand the interplay between collective and individual decision-making in this obligate group-raiding predator. I found a clear positive relation between raid size and termite abundance at the foraging site. Furthermore, selectivity of the food source increased with distance. The confirmation of optimal foraging theory suggests that individual scouts must be the main driver behind raid size, choice and raiding behaviour. Therefore most central place foraging behaviours in *M. analis* were not achieved by collective decisions but rather by individual decisions of scout ants. Thus, 1% of the colony (10–20 scouts) decided the fate and foraging efficiency of the remaining 99%.

Division of labour 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 though. Since *M. analis* specializes on a defensive prey, adaptations to reduce their foraging costs can be expected. I 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. Most of the raid 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.

I show that specialization towards a highly defensive prey can lead to very unique adaptations in the foraging behaviour of a species. I explored experimentally the adaptive value of rescue behaviour focused on injured nestmates in social insects. This was not only limited to selective rescuing of lightly injured individuals by carrying them back (thus reducing predation risk) but moreover includes a differentiated treatment inside the nest. These observations will help to improve our understanding of the evolution of rescue behaviour in animals. I further show that most optimal foraging predictions are fulfilled and regulated by a handful of individuals in *M. analis*. Lastly, I 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. All of my observations help to further understand how a group-hunting predator should forage so as to minimize costs while maximizing profits.

ZUSAMMENFASSUNG

Ein effizientes Furagierverhalten ist eine der wesentlichsten Voraussetzungen für die Überlebensfähigkeit von Tieren. Die Theorie des „Optimal Foraging“ versucht, das Furagierverhalten durch die übergreifende Frage zu verstehen: Wie sollten Tiere nach Futter suchen/jagen, um die Kosten zu minimieren und gleichzeitig die Gewinne zu maximieren? Soziale Insekten, die fast jede natürliche Nische durch diverse Strategien besetzt haben, bieten sich als ideale Gruppe an, um zu untersuchen, wie gut „Optimal Foraging“ ihr Verhalten und ihren Erfolg erklären kann.

Da Spezialisierung oft zu einzigartigen Anpassungen in Morphologie und Verhalten führt, war das Jagdverhalten von *Megaponera analis* diesbezüglich sehr vielversprechend. Diese Ponerinae Ameisenart ist spezialisiert auf die Jagd von Termiten der Unterfamilie Macrotermitinae an ihren Futterstellen. Ihr Jagdverhalten wird durch eine Handvoll von einzelner Späher (10-20) geregelt, die nach Termiten-Futterstellen suchen, bevor sie zum Nest zurückkehren, um eine große Anzahl von Nestgenossinnen (200-500 Ameisen) zu rekrutieren. Die Ameisen folgen dann dem Späher in einer Kolonne zu den Termiten und zurück, diese Überfälle finden zwei bis fünf Mal am Tag statt.

Es ist wahrscheinlich, dass Prädatoren von defensiver Beute kostenreduzierende Anpassungen entwickeln. Das evolutionäre Wettrüsten zwischen Termiten und Ameisen führte zu verschiedenen Abwehrmechanismen bei Termiten, z.B. eine Soldaten-Kaste, die sich auf die Bekämpfung von Raubtieren spezialisiert hat. Da *M. analis* ein hohes Verletzungsrisiko durch Termitensoldaten hat, haben sich bei ihr einige kostenreduzierende Anpassungen entwickelt. Ich zeige, dass ein einzigartiges Rettungsverhalten bei *M. analis*, bestehend aus verletzten Nestgenossinnen, die zum Nest zurückgetragen werden, die Mortalität reduziert. Diese verletzten Ameisen „rufen“ um Hilfe mit Pheromonen, die in ihren mandibularen Drüsenreservoirs vorhanden sind. Ein Modell, das dieses Rettungsverhalten berücksichtigt, hilft dabei die wichtigsten Faktoren zu identifizieren, welche die Evolution dieses Rettungsverhaltens begünstigen. Ferner wird schwerverletzten Ameisen, die während des Kampfes zu viele Beine verloren haben, nicht geholfen. Interessanterweise wird dies nicht durch den Helfer reguliert, sondern durch die mangelnde Kooperation der verletzten Ameise. Des Weiteren beobachtete ich die Behandlung der Verletzten durch Nestgenossinnen im Nest durch intensives „Allogrooming“/lecken direkt an der Wunde. Eine Unterbindung der Behandlung erhöhte die Mortalität von 10% auf 80% innerhalb von 24 Stunden, höchstwahrscheinlich aufgrund von Infektionen.

Die kollektive Entscheidungsfindung ist einer der Hauptmechanismen bei sozialen Insekten, durch die die Futtersuche reguliert wird. Allerdings spielt die individuelle Entscheidungsfindung, je nach Art des Furagierverhaltens, auch eine wichtige Rolle. In *M. analis* haben nur eine Handvoll von Individuen (die Späher) alle Informationen über die Futterstellen. Ich betrachtete daher die Vorhersagen, die durch „Optimal Foraging“ gemacht werden, um das Zusammenspiel von kollektiver und individueller Entscheidungsfindung bei diesem obligaten Gruppenjäger besser zu verstehen. Ich fand eine klare positive Beziehung zwischen Raubzugsgröße und Termitenabundanz an der Futterstelle. Außerdem erhöhte sich die Selektivität der Futterstelle mit der Entfernung zum Nest. Die Bestätigung der „Optimalen Foraging“ Theorie deutet darauf hin, dass einzelne Späher der Haupttreiber hinter Raubzugsgröße, Wahl und Raubzugsverhalten sein müssen. Dies bedeutet, dass in *M. analis* das Furagierverhalten nicht durch kollektive Entscheidungen, sondern durch individuelle Entscheidungen der Späher reguliert wird. So entschied 1% der Kolonie (10-20 Späher) das Schicksal und die Furagier-Effizienz der restlichen 99%.

Die Arbeitsteilung ist einer der Hauptgründe des Erfolgs sozialer Insekten. Arbeiterpolymorphismus, Alterspolyethismus und Arbeitsteilung bei primitiveren Ameisen, wie den Ponerinen, blieben bisher jedoch meist unerforscht. Da *M. analis* sich auf eine defensive Beute spezialisiert hat, sind Anpassungen zur Reduzierung ihrer Furagierkosten zu erwarten. Ich zeige, dass die Arbeitsteilung und Kolonnenformation während der Jagd viel anspruchsvoller ist, als bisher angenommen. Die Kolonnenformation war bemerkenswert stabil: dieselben Ameisen nahmen ähnliche Positionen in späteren Raubzügen ein und die vorderen Ameisen kehrten sogar zu ihrer Position zurück, wenn diese absichtlich verändert wurde. Dies weist auf unbekannte Regulationsmechanismen für die Bildung der Kolonne hin. Darüber hinaus wurden die meisten der Raubzugsaufgaben nicht von vorgegebenen Mitgliedern des Raubzugs ausgeführt, sondern wurden je nach Bedarf während der Jagd verteilt.

Meine Versuche zeigen, dass die Spezialisierung auf eine hoch defensive Beute zu sehr einzigartigen Anpassungen im Furagierverhalten einer Art führen kann. Ich erforschte experimentell den adaptiven Wert eines Rettungsverhaltens, das auf verletzte Nestgenossinnen bei sozialen Insekten fokussiert war. Dies beschränkte sich nicht nur auf

die selektive Rettung von leicht verletzten Individuen, welche zurückgetragen wurden (wodurch das Prädationsrisiko reduziert wurde), sondern umfasst darüber hinaus eine differenzierte Behandlung im Nest. Ich zeige weiter, dass die meisten „Optimal Foraging“ Vorhersagen von einer Handvoll Individuen in *M. analis* erfüllt und reguliert werden. Schließlich postuliere ich die Hypothese, dass der kontinuierliche allometrische Größenpolymorphismus in *M. analis* eine größere Flexibilität bei der Aufgabenverteilung ermöglicht, die aufgrund der Unberechenbarkeit der Aufgabenanforderungen in einem unregelmäßigen System wie dem Jagen von Termiten in Gruppen Erforderlich ist. Alle meine Beobachtungen verbessern unser Verständnis des Verhaltens eines Gruppenjägers, das während der Jagd die Kosten zu minimieren und die Gewinne zu maximieren hat.

A *Megaponera analis* raid on the way out to the termites

CHAPTER I: GENERAL INTRODUCTION

Optimal foraging

After reproduction, efficient foraging is one of the most important traits for the survival and overall fitness of animals (MacArthur & Pianka 1966). The theory of optimal foraging tries to explain foraging patterns we observe in animals through various hypotheses (MacArthur & Pianka 1966, Olsson et al. 2008, Pearce-Duvet et al. 2011). With the main question being: How should animals forage so as to minimize costs while maximizing profits? There is of course no universal answer, depending on the natural history of the species and the food it eats the answer to this question varies greatly. From this overarching theory various hypothesis have developed, such as marginal value theorem (Charnov 1976), abundance prediction (Schöner 1971, Bailey & Polis 1987), maximization of energy (Schöner 1971) and hunger state prediction (MacArthur & Pianka 1966, Schöner 1971), for details see Chapter III: Introduction.

Animals that have to return to a central place (like a nest) necessitate further considerations (Orians & Pearson 1979, Schöner 1979). In birds, and many mammals, this is the case at one essential stage of their life: the rearing of brood (Vaughan et al. 2010, Gill 2006). In other species it is an obligate condition throughout most of their life, like in eusocial animals such as ants and termites (Hölldobler & Wilson 1990). It is therefore necessary to take into account travel time when trying to understand their foraging patterns: central place prediction theory (Orians & Pearson 1979).

Applying optimal foraging theory predictions on animals helps us to better understand their behaviour. It is vital for the conservation of a species and generally for developing a better understanding of their natural history (Schöner 1971, MacArthur & Pianka 1966, Olsson et al. 2008, Pearce-Duvet et al. 2011). Social insects are distinct within the animal kingdom. They have colony sizes of dozens to millions of individuals, while the colony as a whole still has to be considered one super organism (Hölldobler & Wilson 1990). The flexibility of assigning different tasks to differing numbers of individuals made them an interesting model organism for optimal foraging theory (Hölldobler & Wilson 2008).

Ants (Formicidae)

Ants are considered to be one of the most successful families in the animal kingdom (Hölldobler & Wilson 1990). They occur on all continents, except Antarctica, and there are estimated to be around 22.000 species (Agosti & Johnson 2003). This high diversity of species in widely different habitats led to various different foraging strategies and life cycles.

Division of labour is one of the main reasons for their success (Hölldobler & Wilson 1990). The ability to separate reproduction (queen) from foraging (workers), allowed them to conduct far riskier foraging strategies (for the individual worker) without risking the reproductive success of the colony (fitness). In many species division of labour goes further than just reproduction (Hölldobler & Wilson 2008). Worker division of labour can include brood care and other nest tasks or going out to forage. This is generally regulated through worker age, morphology and dominance interactions (Hölldobler & Wilson 2008). This allowed for the rise of various different life strategies. In fungus growing ants for instance division of labour has a morphological basis with the work generally being partitioned by size, i.e. smaller workers handle smaller larvae and food (Wilson 1980). Another efficient method to maximize worker efficiency is division of labour by age. The older a worker gets the riskier the tasks it conducts should be to maximize life expectancy in the colony as a whole (Hölldobler & Wilson 2008). Thus young ants generally conduct brood care and other nest tasks before moving on to forage outside the nest as they get older.

Foraging strategies in ants can generally be divided into three main categories (although there are many exceptions) (Traniello 1989).

(1) Solitary foraging (diffuse foraging): workers search for food alone outside the nest. This strategy is generally used in more opportunistic predatory or scavenger species that can overwhelm prey individually, like trap jaw ants (*Odontomachus*) (Raimundo et al. 2009) or desert ants (*Cataglyphis*) searching for small/dead insects (Cerdá 1988).

(2) Group foraging: workers leave the nest in large groups searching and subduing prey as they encounter them. This for instance is the case in army ants, were millions of individuals leave the nest together, thereby covering large areas and killing prey which a sole individual would not be able to (like larger animals or colonies of other social insects) (Hölldobler & Wilson 1990).

(3) Recruitment: In this foraging strategy work is mainly divided into two main categories, scouts and recruits. Scouts search for food (similar to solitary foraging ants) but instead of fully exploiting the food the scouts go back to the nest to recruit nestmates to the location of the food. This is a very widespread strategy in ants and can range from scouts recruiting a handful of individuals to large mass recruitments of hundreds of individuals. This strategy is especially beneficial when foraging in a heterogeneous environment were food sources are encountered rarely but in high concentrations, making individual exploitation less efficient (MacArthur & Pianka 1966, Longhurst et al. 1978).

Group recruiting ant species offer themselves as an ideal group to study optimal foraging theories. How can a colony as a whole maximize its energy intake when only a few individuals (scouts) hold all the information on the food sources? In ants that create pheromone trails to long lasting food sources (like honey dew) this can be regulated passively/collectively over time through pheromone trail reinforcement and is relatively well studied (Hölldobler & Wilson 2008). In ant species in which scouts recruit a large number of individuals to a food source for a single event (i.e. a hunt) the mechanisms are not as evident.

Matabele ants (*Megaponera analis*)

Megaponera analis is a specialized termite-predating ant species found in sub-Saharan Africa (Fig. I.1) (Schmidt & Shattuck 2014). The species has caught the attention of various scientists due to its very pronounced continuous size polymorphism, a rare phenomenon in ponerines (Crewe et al. 1984, Villet 1990), and its foraging strategy of only group-hunting termites of the subfamily Macrotermitinae (Longhurst et al. 1978, Yusuf et al. 2014).

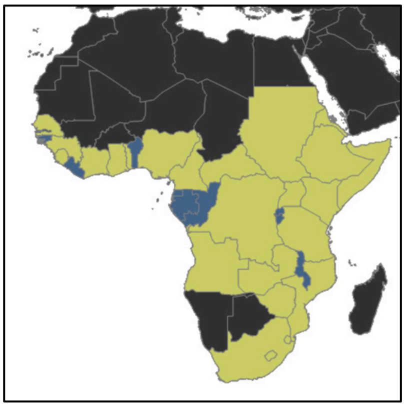

Fig. I.1. Africa distribution map of *Megaponera analis*. Green: present; Blue: likely present; Black: absent. As taken from Schmidt and Shattuck (2014).

Megaponera analis has a clear work division inside the nest, by partitioning their work by size: the smaller workers take 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: younger workers conduct nest tasks and older workers go out to forage and scout (Villet 1990).

The foraging pattern starts with 10–20 scouts leaving the nest to search for termite foraging sites (Bayliss & Fielding 2002, Longhurst & Howse 1979). The searching phase can last for up to one hour and cover an area of up to a 50m radius around the nest; if the scout is unsuccessful it will then return to the nest by a circuitous route (Bayliss & Fielding 2002). Once a scout finds a potential termite site it will start investigating it, while avoiding contact with the termites (Bayliss & Fielding 2002, Longhurst & Howse 1979). Afterwards the scout will return to the nest to recruit nestmates to conduct a raid. The number of nestmates recruited for a raid can vary between 100 and 600 individuals and the scout will lead them in a column formation to the termites (Bayliss & Fielding 2002, Longhurst & Howse 1979). At the hunting ground division of labour occurs: the larger ants (majors) break open the protective soil cover created by the termites while the smaller ants (minors) rush into these openings to kill and pull out the prey (Corbara & Dejean 2000). The hunting phase lasts between 5–20 min depending on how fast the termites are able to retreat into their galleries (Yusuf et al. 2014). Afterwards the dead termites get collected by the larger ants, the column forms again and the raiding party returns back to the nest in the same column formation. These raids occur 2–5 times per day in the morning and evening hours (Bayliss & Fielding 2002, Yusuf et al. 2014) and due to the very conspicuous raids gave them the trivial name Matabele ants, after a warmongering tribe in southwest Africa (Wilson 2014).

These continuous raids throughout the year have not only let to adaptations in the ants foraging behaviour, but also in its prey: the termites.

Termites

Optimal foraging theory does not just apply to the predator but also of course to its prey. Unlike ants, termites have specialized on feeding exclusively on dead plant matter (through symbiosis with fungi or gut microbes) (Bignell et al. 2011). This forces them to be rather stationary during foraging on leaf litter or dead wood, making them relatively easy prey. Termites are the main prey for various different predators, of which ants play a major role

(Sheppe 1970). To minimize costs during foraging termites therefore developed various different strategies to minimize mortality (Prestwich 1984). Macrotermitinae are considered the higher termites within the termite family (Bignell et al. 2011). They are generally distinguished by foraging from a central place (nest) in the surrounding area (in contrast to other termite genera which live in the same place they forage, like dead wood) (Bignell et al. 2011). Another major adaptation is the development of a soldier caste, which have strong sclerotized heads and mandibles (Prestwich 1984). These soldiers form the main defensive force both at the termite foraging sites and in the nest. In many species these soldiers can be divided up into two categories: minors and majors (Prestwich 1984). The minors often stand as sentries at the surrounding of the foraging site ready to alarm nestmates of potential threads by “head banging” on the ground, thereby sending vibrational cues for retreat to the workers (Connétable et al. 1999). The larger soldiers on the other hand primarily are there to fend off larger predators and are capable of inflicting serious injuries on insects and even mammals (Prestwich 1984).

Evolutionary Arms Race

These continuous predator-prey interactions are a classic example of an evolutionary arms race (Dawkins & Krebs 1979). While the termites developed better ways to defend and warn themselves from predators, the predators had to become more efficient in their foraging process to sustain these increased costs. Scouts of *M. analis* therefore had to find termite foraging sites without warning the termites of their presence. At the same time raids had to be efficient enough to kill termites in large numbers before they were capable of retreating into their galleries. Furthermore *M. analis* had to somehow reduce the costs of hunting prey able to inflict serious injuries on their workforce.

Rescue behaviour and social immunity

There are some examples on how ants are able to reduce their foraging costs when facing increased mortality risks. Ant species that forage in environments with high antlion concentrations suffer of increased forager mortality (Hollis & Nowbahari. 2013b). The ants therefore developed a rescue system with the trapped ants “calling” for help, thereby attracting nestmates to pull them out of the antlions embrace (Nowbahari et al. 2009). It has also been observed in *M. analis* that during fights against army ants (*Dorylus* sp.), clinging army ants were removed from nestmates during and after the fight (Beck & Kunz 2007). Furthermore, injured *M. analis* raiders frequently get carried back to the nest by nestmates potentially thereby also reducing their mortality (Burgeon 1929, Yusuf et al. 2014).

If nestmates that lost extremities by termite soldiers are to truly recover from their injuries, treatment is expected to occur inside the nest. Social insects are especially prone to infections due to the low genetic diversity within a colony and the frequent contacts between individuals facilitating transmission (Cremer et al. 2007). Positive social interactions between individuals are therefore expected to compensate these deficits beyond the single individual immune competence: social immunity (Cremer et al. 2007, Meunier 2015). This has been extensively studied in ants infected by fungus or other parasites (Fernandez et al. 2006, Cremer et al. 2007, Tranter et al. 2015, Theis et al. 2015, Tragust 2016), but never in ants that suffered severe injury.

Aims and Study design

The main overarching question was how did the evolutionary arms race between *M. analis* and termites lead to adaptations in the foraging behaviour of *M. analis* so as to maximize its net energy gain.

I therefore explored how the ants were capable of coping with the increased injury risk involved when hunting defensive prey (Chapter II). I discovered that the ants had developed a full rescue system, with injured ants (in the form of lost extremities) at the hunting ground “calling” for help with pheromones and being carried back to the nest. I therefore analysed how these injured ants were capable of recovering inside the nest and if the ants were able to distinguish between lightly and heavily injured ants (Chapter III).

I further wanted to know how well optimal foraging theory could explain the foraging patterns and variations in raid size and distance (100-600 ants, 1-50 m). If this was the case, how was it regulated/communicated when only 1% of the colony (the scouts) held all the vital information about the foraging sites (Chapter IV). Lastly the rather unique group-raiding behaviour of *M. analis* made me wonder how task allocation worked when the necessity of some tasks could only be determined during/after the hunt, due to the unpredictability of termite yield at the foraging sites (Chapter V).

To study these questions I spent 27 months in the Comoé National Park, Côte d'Ivoire (Ivory Coast), at the Comoé NP Research Station located in the park. The station allowed me to conduct field and controlled lab experiments directly in the park (Fig. I.2).

Fig. I.2. Drone picture of the Comoé National Park Research Station.

Megaponera analis worker with two clinging *Pseudocanthotermes militaris* termite soldiers after the hunt

CHAPTER II: RESCUE BEHAVIOUR

As published in: Frank ET, Schmitt T, Hovestadt T, Mitesser O, Stiegler J, Linsenmair KE (2017). Saving the injured: Rescue behavior in the termite-hunting ant *Megaponera analis*. *Science Advances* 3:e1602187

ABSTRACT

Predators of highly defensive prey likely develop cost reducing adaptations. The ant *Megaponera analis* is a specialized termite predator, solely raiding termites of the subfamily Macrotermitinae at their foraging sites. The evolutionary arms race between termites and ants led to various defensive mechanisms in termites, e.g. a caste specialized in fighting predators. As *M. analis* incurs high injury/mortality risks when preying on termites, some risk mitigating adaptations seem likely to have evolved. Here we show that a unique rescue behaviour in *M. analis,* consisting of injured nestmates being carried back to the nest, reduces combat mortality. After a fight injured ants are carried back by their nestmates, these ants have usually lost an extremity or have termites clinging to them and are able to recover within the nest. Injured ants, which are forced to return without help, die in 32% of the cases. Behavioural experiments show that two compounds, dimethyl disulphide and dimethyl trisulphide, present in the mandibular gland reservoirs, trigger the rescue behaviour. A model accounting for this rescue behaviour identifies the drivers favouring its evolution and estimates that rescuing allows for maintaining a 28.7% larger colony size. Our results are the first to explore experimentally the adaptive value of this form of rescue behaviour focused on injured nestmates in social insects and help us identify evolutionary drivers responsible for this type of behaviour to evolve in animals.

INTRODUCTION

Helping behaviour has been observed throughout the animal kingdom, from social insects to primates (Hollis & Nowbahari 2013a). Rescue behaviour observed in ants can emerge from predator prey interactions, by rescuing nestmates that have fallen into an antlion trap by digging, pulling the ant out and attacking the antlion or excavating ants trapped under sand or soil (Hangartner 1969, Nowbahari et al. 2009, Hollis & Nowbahari 2013b, Hollis et al. 2015). All hitherto observed types of rescue behaviour in social insects were always directed towards individuals under an imminent threat (Hölldobler & Wilson 1990, Hollis & Nowbahari 2013a, Taylor et al. 2013), i.e. suffocation or being eaten.

Megaponera analis is a strictly termitophagous ponerine ant species found in sub-Saharan Africa from 25°S to 12°N (Schmidt & Shattuck 2014), specialized on raiding termites of the subfamily Macrotermitinae at their foraging sites (Levieux 1966, Longhurst et al. 1979, Lepage 1981, Bayliss & Fielding 2002, Yusuf et al. 2014). A scout ant that has returned to its nest after having found an active termite foraging site initiates a raid. It will recruit approximately 200-500 nestmates and lead them to the termites in a column like march formation, which can be up to 50 meters away from the nest (Longhurst & Howse 1979, Bayliss & Fielding 2002, Yusuf et al. 2014). During the raid division of labour occurs (Corbara & Dejean 2000); larger ants (majors) break open the protective soil cover created by the termites while the smaller ants (minors) rush into these openings to kill and pull out the prey (Villet 1990). Afterwards the majors collect the dead termites, the column forms again and the hunting party returns to the nest. These raids occur 2-4 times a day (Levieux 1966, Longhurst et al. 1978, Lepage 1981, Bayliss & Fielding 2002, Yusuf et al. 2014). Termites have evolved various ways to defend themselves effectively against predators like *M. analis*, of which a specialized soldier caste with strong sclerotized heads and big mandibles is the main defensive force (Sheppe 1970, Prestwich 1984). Consequently ants involved in the hunting process incur high injury risks.

We observed a unique helping behaviour in *M. analis* to compensate for this high injury rate by carrying back injured ants to the nest. The carrying of ants after the hunt was also observed in Kenya (Yusuf et al. 2014) and the Democratic Republic of Congo (Burgeon 1929); however, no attempt was made in those studies to explore the adaptive value of this behavior to the colony or the individual. We further observed removal of termites still clinging on to extremities in the nest and rescue behaviour towards ants that carry long-term injuries in the form of lost extremities. Such specialized rescue behaviour is unanticipated in insects, were the value of individuals is generally underestimated, and could provide further proof that empathy is not necessary for helping behaviour to emerge in animals (Vasconcelos et al. 2012).

MATERIALS AND METHODS

Experimental design

The study was conducted in 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) (Konaté & Kampmann 2010). Observations throughout several days 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 (Longhurst & Howse 1979, Bayliss & Fielding 2002, Yusuf et al. 2014). Night raiding was also observed, but was not included in this study. Experiments and observations were 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 November 2015. *Megaponera analis* is found throughout sub-Saharan Africa from 25°S to 12°N (Schmidt & Shattuck 2014). We observed *M. analis* in a total of 52 different colonies for a total of 420 raids on which the predominantly hunted termite species was *Pseudocanthotermes* sp.. Living nests of *Macrotermes bellicosus*, which in other areas were often favoured prey, could potentially cause a higher and more fatal injury rate due to their stronger soldiers; this species was absent in the vicinity of the study area. Colony size for 10 excavated colonies was between 900-2300 ants, a result comparable to previous studies in other regions (Villet 1990, Yusuf et al. 2013). 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 majors (head width > than 2.40 mm), minors (head width < 1.99 mm) and intermediates (head width 2.40 - 1.99 mm) as proposed by Villet (1990).

Quantification of carried ants

The experiments and observations were conducted in the field, by waiting in front of a colony for a raid to be initiated and then following the raid column to the hunting ground.

In total we observed 420 raids in 52 different colonies. These 420 raids were used in various different experiments and observations.

To quantify the number of ants being carried back from the hunting ground we counted the number of ants carrying a nestmate during the return journey shortly before arriving at the nest in a total of 53 raids.

To classify the type of injuries the carried ants had (i.e. categories: lost limb, unharmed and termite clinging) we retrieved them with forceps and investigated them. This was done in 20 raiding columns of 20 different colonies for a total of 154 carried ants (Fig. II.1*A* and Fig. II.S1*B*).

To quantify the number of long-term injured ants participating in raids we collected ants of all castes from 20 raids, each from a different colony, when raid columns were leaving the nest (i.e. before any new fight could have taken place); in total we collected 763 minors, 582 majors and 502 intermediates (total *N*= 1847, Fig. II.4).

Velocity and mortality

This experiment was conducted 20 times for each of the 3 categories (lost limb, termite clinging and carried unharmed) in individual raids with an additional control test of healthy individuals (Fig. II.1*A,B*). Individuals for the experiments were randomly selected from the pool of carried ants in a raid, with the control being a healthy ant walking unassisted in the returning raid column showing no sign of injury or handicap. Velocity was measured for the distance the ant followed the pheromone trail back to the nest. If a predator killed the ant during the return journey the speed was calculated based on the distance covered up to that incident. This allowed us to quantify the handicap and mortality risk each injury posed during the return journey (Fig. II.2*A-E*). If the ant stopped moving during the return journey, most likely due to fatigue, the time was also stopped and the velocity calculated up to that point.

Injury recovery

To analyse the potential recovery of ants that lost an extremity we randomly cut off one leg on each side of a healthy ant (with scissors), picked up during the return journey of the raid. The ant was then released on the return pheromone trail and the covered distance in 60 seconds was measured. This experiment was repeated with the same individual 24 hours later and with healthy individuals as a control. It was conducted with laboratory colonies so we could easily reproduce similar conditions concerning the time between experiments and nest conditions. For individual recognition acrylic marker pens were used, since no indication of any lasting disturbance by the marking was found in prior observations (Fig. II.S2).

Ethogram of rescue behaviour

Since there was no significant difference in the quantity of ants helped at the hunting ground or on the return journey (Fig. II.5*B*, Fisher's exact test: P=0.33, N=20) the subsequent experiments (Fig. II.5*A* and *C*) were carried out during return journeys for easier reproduction of trials. The experiments were repeated 20 times with at least 5 different colonies involved, with each return raid being used for only 1 trial. For these experiments an injured ant (or dummy) was placed at the front of the return column at least 1 m away from the hunting ground. All behavioural reactions by the nestmates were recorded until the entirety of the column passed the study subject. The behavioural reactions of the helping ants consisted of five categories: 1. Ignored: Contact with the study subject was less than 2 seconds; 2. Investigated: The study subject was antennated for more than 2 seconds; 3. Picked up: The study subject was fully lifted from the ground; 4. Carried back: The study subject was carried back for at least 20 cm towards the direction of the nest; 5. Carried away: The study subject was removed from the return column in a direction that was not the one back to the nest, i.e. away from the column. For graphical illustration and statistical analysis we summarized behaviours 3 and 4 as rescue behaviour and 3 in combination with 5 as disposing of the study subject.

Laboratory colonies

A total of 10 colonies were excavated and placed in artificial nests in the field stations lab (colony size 1373±520 ants). Consisting of a 20x20x10 cm large nest made of PVC connected to a 1x1m arena. For raids this arena was connected by a 10m long corridor to a second arena (1x1m). The ground was covered with earth from the surrounding area. In the second arena *Macrotermes bellicosus* termites were placed, which were collected from the surrounding area with a pot filled with dry grass. These termites were found by scouts and triggered raiding behaviour on which we performed the injury recovery experiments. For further details on lab keeping see Yusuf et al. (2013).

Pheromone & Stridulatory Communication

To inhibit stridulation we coated the stridulatory organ, located between the 1st and 2nd tergite (Hölldobler et al. 1994), with black acrylic colour. After it had 2 minutes time to dry the experiment was conducted. To confirm that stridulation was truly inhibited we triggered normal stridulation behaviour by exposing the ant to CO_2, as previously described by Hölldobler et al. (1994). During this process the sound was recorded with an external microphone, Speedlink SL-8703-BK (Jöllenbeck GmbH, Weertzen, Germany). To visualize the sound a sonogram was created with the digital audio editor Audacity v2.0.5.0 (Fig. II.S1*A*).

For the pheromone experiments we dissected a gland and placed it on a glass surface on which we pushed the thorax of the study subject on, pulling it three times over the burst gland reservoir (for the smaller mandibular gland, two glands were used per experiment). For the experiments with synthetic chemicals we first diluted the substance in hexane until we reached a concentration of 90ng/ml. Subsequently two drops (roughly 9ng of the substance) were applied on a glass surface. The concentrations chosen were similar to the quantities found in a mandibular gland: 14 ng of DMDS and 5 ng of DMTS respectively per gland in a major worker according to Longhurst et al. (1978) and a comparison of the mass spectrometer of the gland reservoir with our solution. After 30 seconds most of the hexane evaporated and the thorax of the dummy was pulled over the glass surface three times.

Chemical analysis

Foraging *M. analis* workers were collected from various colonies at the Comoé National Park (Côte d'Ivoire). The workers were then transported alive to the University of Würzburg (Germany) and killed with CO_2 before excision of the mandibular gland reservoirs. The caput and the mandibles, including the mandibular gland, of 20 ants were then soaked in 1 ml pure pentane for 2 hours (2 caputs and 6 mandibular glands respectively). These extracts were evaporated to a residue of approximately 100 µl. We used 1 µl for GC-MS analyses. These were carried out on a gas chromatograph 6890 coupled to a mass selective detector 5975 from Agilent Technologies (Waldbronn, Germany). The GC was equipped with a DB-5 capillary column (0.25 mm ID × 30 m; film thickness 0.25 µm, J & W Scientific, Folsom, Ca, USA). Helium was used as a carrier gas with a constant flow of 1ml/min. A temperature program from 60°C to 300°C with

5°C/min and finally 10 min at 300°C was employed, with data collection starting 2 minutes after injection. The mass spectra were recorded in the EI mode with an ionization voltage of 70 eV and a source temperature of 230°C.

The software ChemStation (Agilent Technologies, Waldbronn, Germany) for windows was used for data acquisition. Identification of the components was accomplished by comparison with purchased chemicals and the use of a commercial MS database (NIST 4.0). Due to the very small quantities of dimethyl disulphide and dimethyl trisulphide within the extracts we used diagnostic ions and the retention time to confirm the identification.

Rescue behaviour model

We provide an equilibrium model to quantify the possible benefits of rescue behaviour to the colony – the benefit is expressed as the proportional increase in equilibrium worker number of a colony with rescue behaviour compared to a colony that would not show such behaviour. For the sake of argumentation we choose a very simple model that does not account for all the mechanisms that truly regulate worker numbers in ant colonies.

We assume that the worker dynamics of a colony without rescue behaviour is described by equation

$$\frac{dH}{dt} = b - (\varepsilon_H + \mu_0)H \qquad (1)$$

With H the number of non-injured (healthy) workers, b the rate at which new workers are added to the colony, ε_H the rate at which workers are involved in injuring interactions with termites, and μ_0 the base mortality rate of workers.

For such a colony, colony size (worker number) will settle into equilibrium:

$$\hat{H} = \frac{b}{\varepsilon_H + \mu_0} \qquad (1b)$$

A colony that manages to rescue a fraction f $(0 \leq f \leq 1)$ of the workers injured in action will in addition build a 'pool' J of workers that were injured in previous raids but rescued; conservatively we do not separate between injured ants that may ultimately recover (and would thus return to pool H) and workers that carry permanent damages such as a lost extremity. The dynamics of injured ants is described as

$$\frac{dJ}{dt} = f\varepsilon_H H - (\mu_J + \mu_0)J \tag{2}$$

With f ($0 \le f \le 1$) the proportion of ants injured in combats that survive and μ_J the added (future) mortality rate of injured compared to non-injured workers. For simplicity and based on empirical observation we, conservatively, assume that a second injury carried away in another raid would always be fatal. The equilibrium number of injured ants in a colony is thus

$$\hat{J} = \hat{H}\frac{f\varepsilon_H}{\mu_J + \mu_0} \tag{2b}$$

The relative size of colonies with rescue behaviour compared to one not showing such behaviour, i.e. a total loss of injured individuals (f=0), is thus defined by

$$\frac{\hat{H} + \hat{J}}{\hat{H}} = 1 + \frac{f\varepsilon_H}{\mu_J + \mu_0} \tag{3}$$

Quantification of Model

The observed survivability of an injured ant not receiving help is 68% (Fig. II.2*A*). $f = 0.68$ thus characterizes hypothetical colonies without rescue behaviour, while in colonies were the behaviour is present $f = 1$, since all rescued ants were observed in later raids. All other parameters stay the same in both cases and were calculated as follows. Since we can only quantify the injury ratio in the colony for ants that lost an extremity our value ε_H was defined as the percentage of lost limb injuries per raid (0.21*3) per day (3) divided by the ratio of healthy ants in a colony (0.79*1373, *N*=10 excavated colonies), therefore ε_H=0.0017. We conservatively argue that the added mortality of a previously injured ant is the probability of getting injured again, therefore $\mu_J = \varepsilon_H$, in our scenario. We estimated the birth rate *b* of the colony by observing the callow worker population of excavated nests until they were fully sclerotized (106 ± 30 callow workers per excavated colony, *N*=5). Sclerotization time was calculated to be 8 days on average (*N*=5). Leading us to an estimate of 13.3 ± 3.8 ants born per day. Inserted in formula 1b we were thereby able to calculate μ_0 = 0.0076. To test the precision of the parameters estimated from empirically observed data we compared the empirical ratio of injured ants in the colony (0.21, Fig. II.4) to our model prediction from equation 2b: 0.21. The good agreement of predicted and empirical values allows us to reliably calculate the benefit of the rescue behaviour, by comparing the calculated colony size (H+J) of a colony with rescue behaviour to one without. Our results indicate that the helping behaviour results in a 6.0% larger colony size if we just consider the benefit for the 21% of carried ants that lost an extremity (Fig. II.1*A*). If extrapolated for all injuries the benefit of the rescue behaviour can be estimated to be 28.7%.

Statistical analysis

For statistical analysis and graphical illustration we used the statistical software R v3.1.2 (R Core Team 2013) with the user interface RStudio v0.98.501. 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 with a Tukey HSD test 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 Bonferroni correction. To analyse the ethogram data a Fisher's exact test with Bonferroni correction was used with a no help control (0 out of 20 helped) compared to our treatments. Median values mentioned in the text are followed by a median absolute deviation.

RESULTS

Injured *M. analis* ants were antennated by their nestmates at the hunting ground whereupon they adopted a pupal pose, most likely for ease of transportation back to the nest (Fig. II.S1*A*). On an average raid, a median of 3 ± 2.9 ants out of 416 ± 153 were carried back (N=53 raids with 154 carried ants), accumulating to 9-15 rescued ants per day (3-5 raids per day). In only 11% of the raids no ants were carried back (6/53) and in half of those cases the raid itself was unsuccessful (no encounter with termites at the hunting ground). If we consider a mean estimated birth rate of 13.3 ± 3.8 ants per day (N=5; for estimate calculation see materials & methods section: quantification of model), the rescued ants make up a large proportion of the daily turnover in the colony.

Value of rescue behaviour for the individual

We classified carried ants into three mutually exclusive categories: (i) ants that partially or completely lost an extremity (antenna or leg), (ii) ants that have termites clinging onto their bodies and (iii) ants that appear to carry no obvious injury (Fig. II.S1*B*). The majority of carried ants had a termite clinging on an extremity (Fig. II.1*A* and Table II.S1). This handicap reduces the speed of the ant the most (4.5% of the mean speed of a healthy individual; Fig. II.1*B* and Table II.S1) and, if removed successfully, had no long-term consequences.

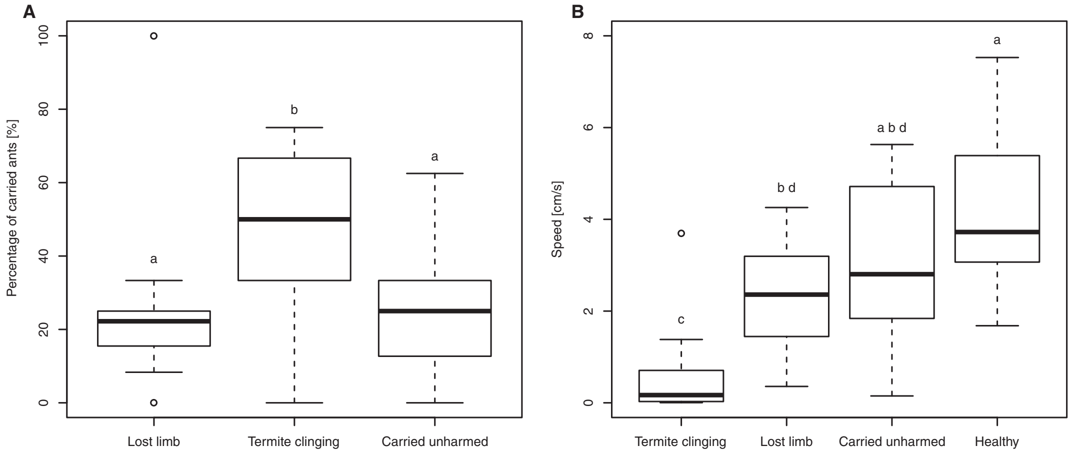

Fig. II.1. Injury type frequencies and handicap in injured *M. analis* ants. Box-and-whisker plot showing: median (horizontal line), interquartile range (box), distance from upper and lower quartiles times 1.5 interquartile range (whiskers), outliers (> 1.5X upper or lower quartile) and significant differences (different letters) for **(A)** distribution of different injury types being carried by helper ants; Lost limb: ant that lost one or more legs or antennas; Termite clinging: ant that still had a termite clinging onto its body; Carried unharmed: ant that appears unharmed to the naked eye (Kruskal-Wallis rank sum test followed by a Dunn's test with Bonferroni correction; *N*=20 trials with 20 colonies with a total of 154 helped ants). **(B)** Running speed of ants affected by different injuries and healthy individuals as control (Healthy) (Kruskal-Wallis rank sum test followed by a Dunn's test with Bonferroni correction; *N*=20 trials with 20 colonies). See also Table II.S1 for detailed statistical results and Fig. II.S1 for illustration of injury types.

When 20 randomly selected individuals from each of the three categories of carried ants were forced to return alone from the hunting ground, 32% (*N*=19/60) of them died (Fig. II.2*A*), in contrast to 10% of healthy individuals (*N*=2/20). Ants that were carried back to the nest were never observed to be under any threat of predation (*N*=420 raids observed during the entire field phase), thereby reducing return journey mortality of injured ants from 32% to close to 0%. The main cause of death when forced to return alone was predation by spiders (57.1%: *N*=12 out of the 21 ants killed during the return journey alone from the hunting ground, Fig. II.2*B*-*E*). Ants that had a termite clinging onto an extremity had the highest mortality rate (50%, *N*=10/20, Fig. II.2*A*). In nature injured individuals were never observed to return alone without help but 6 fatal injuries were observed at the hunting ground (in a total of 53 raids): removed head, thorax, gaster or multiple legs. These ants were left behind at the hunting ground.

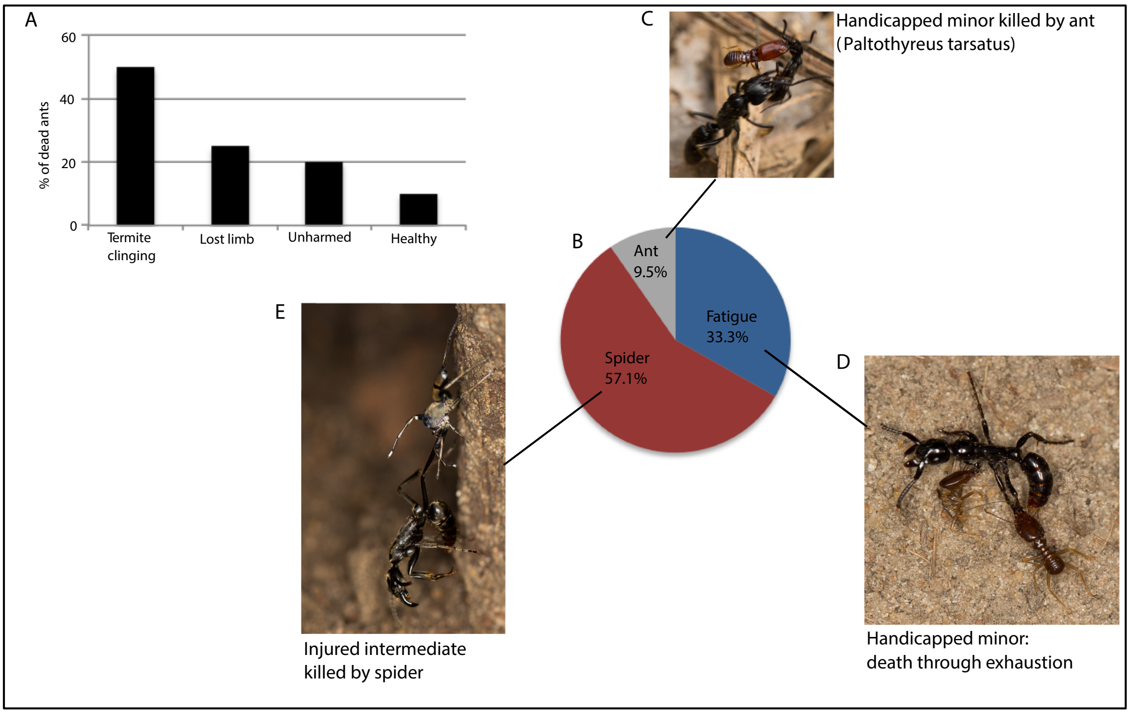

Fig. II.2. Mortality and predation of injured and handicapped individuals if not rescued. (A) Percentage of injured and handicapped ants dying during the return journey for the three classified carried ant types and control (Termite clinging, lost limb, unharmed, healthy) if not helped (*N*=20 for each type, total *N*=80). **(B)** Percentages of the different mortality causes during the return journey (*N*=21 out of 80 died); spider (red): killed by a predatory spider, fatigue (blue): ant stops moving during return journey, most likely due to exhaustion; ant (grey): injured minor carried off/killed by another ant. **(C)** Handicapped minor with termite clinging onto extremity carried off by a forager of *Paltothyreus tarsatus*. **(D)** Handicapped minor with 2 termite soldiers clinging onto extremities stops moving due to exhaustion after a 52-minute return journey. **(E)** Intermediate with a lost extremity returning alone from the hunting ground ambushed by a Salticidae (jumping spider).

Ants that were carried back to the nest were observed again in subsequent raids 95% of the time (*N*=38/40), sometimes less than an hour after the injury (individuals were marked with acrylic colour codes for recognition). Termites clinging onto extremities were removed in 90% of the cases in the following 24 hours without removing the extremity (*N*=20), thereby completely rehabilitating the handicapped individual. Ants that had lost two randomly selected legs were able to recover in the safe confines of the nest. Twenty-four hours after their injury they reached mean running speeds 32.1% faster than freshly injured ants, a speed not significantly different from that of healthy individuals (Fig. II.3 and Table II.S2).

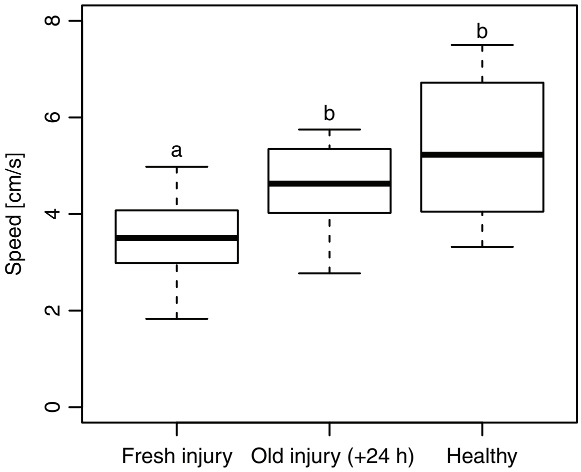

Fig. II.3. Speed of injured ants at different times after injury. Box-and-whisker plot showing: median (horizontal line), interquartile range (box), distance from upper and lower quartiles times 1.5 interquartile range (whiskers) and significant differences (different letters) of the different running speeds 5 minutes after removing two legs (Fresh injury), 24 hours later (Old injury (+24h)) and of healthy ants (Healthy) (Kruskal-Wallis rank sum test followed by a Dunn's test with Bonferroni correction; *N*=20 trials with 5 colonies). See also Table II.S2 for detailed statistical results.

96.1% of the carried ants were minors (*N*=154 in 20 observed raids). This is also reflected by the fraction of injured individuals in raiding columns before the fight. A significantly larger fraction of intermediates and minors had lost an extremity compared to majors (Fig. II.4 and Table II.S3). The few majors that were carried either had a termite clinging on them or had lost an extremity, they never appeared unharmed.

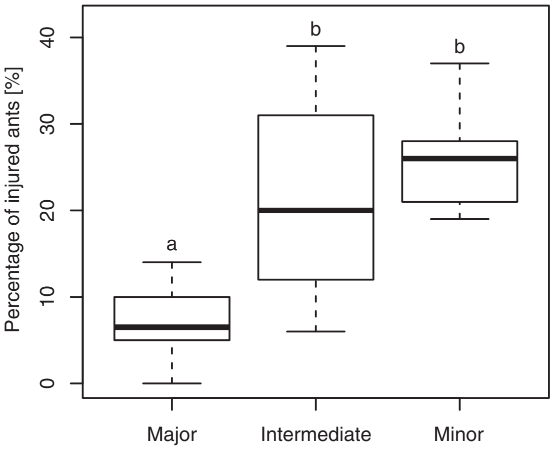

Fig. II.4. Distribution of long-term injuries in different size classes of *M. analis*. Box-and-whisker plot showing: median (horizontal line), interquartile range (box), distance from upper and lower quartiles times 1.5 interquartile range (whiskers) and significant differences (different letters) for the percentage of ants that lost an extremity in previous raids for majors, intermediates and minors (ANOVA followed by Tukey HSD; n=20). See also Table II.S3 for detailed statistical results.

Focus of rescue behaviour

To show that this behaviour is indeed focused on injured nestmates we artificially injured individuals by removing one leg on each side. These individuals were then placed at the front of the return column, forcing all ants in the column to walk past the injured individual. While healthy and dead individuals were ignored or disposed of by their nestmates, the artificially injured individuals were picked up and carried back to the nest (Fig. II.5*A*; Table II.S4). Artificially injured individuals from other colonies were always attacked and removed from the column (Fig. II.5*A* and Table II.S4).

Rescue behaviour occurred both directly at the hunting ground and on the return journey, whereas artificially injured ants on the way to the termites were ignored (Fig. II.5*B* and Table II.S4).

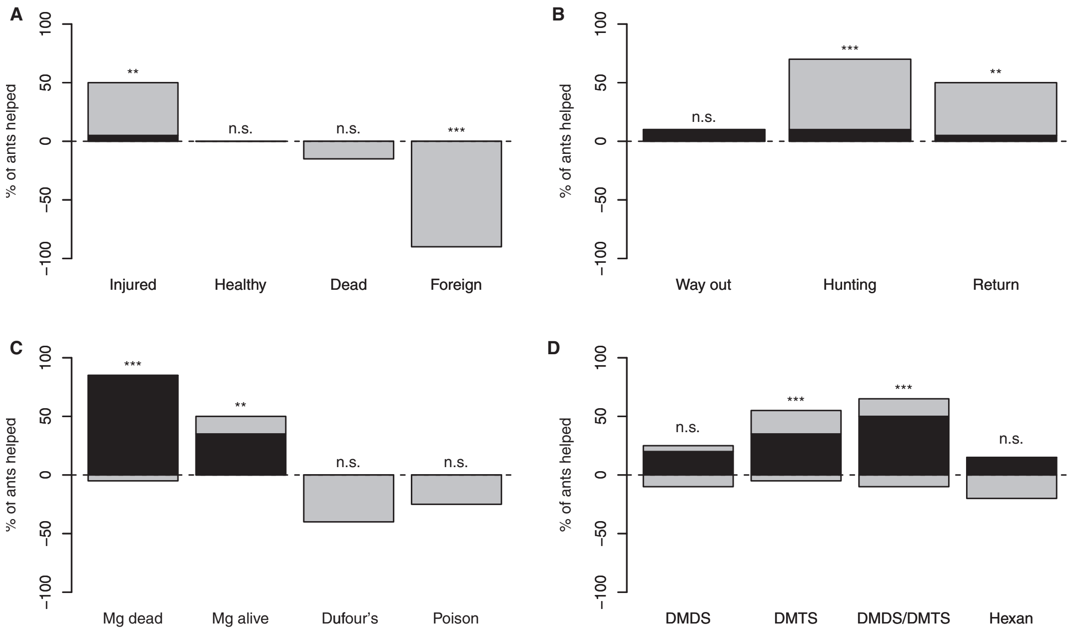

Fig. II.5. Behavioural responses of helper ants towards different treatments of injured individuals or dummies. Positive values show clear attempts of help by picking up the ant and dropping it again (black) or carrying it back to the nest (grey). Negative values show behaviour in which the ant was disposed of (dragged away from the raiding column or attacked) (Fisher's exact test for count data between neutral treatment (Healthy) and the other categories; *N*=20). **(A)** Response towards different injury states. **(B)** Response at different points of the raid (Way out: On the way towards the termites; Hunting: at the hunting ground; Return: on the return journey after the fight). **(C)** Response towards dummies (dead minors) treated with different glands (Mg dead: mandibular gland applied on a dummy; Mg alive: mandibular gland applied on a healthy/living ant; Dufours: Dufour's gland applied on a dummy; Poison: poison gland applied on a dummy). **(D)** Response towards dummies treated with different synthetic compounds (DMDS: dimethyl disulphide, DMTS: dimethyl trisulphide, DMDS/DMTS: 50/50 mixture of DMDS and DMTS, Hexane: pure hexane as control). See also Table II.S4 for detailed statistical results.

Gland and pheromone triggering rescue behaviour

When looking for the signal triggering this rescue behaviour we first ruled out stridulation (Hölldobler et al. 1994), a mechanism known to trigger helping behaviour in other ants (Hollis et al. 2013, Hölldobler & Wilson 1990). We observed artificially injured ants, on which stridulation was inhibited, to still be rescued ($P<0.001$, $N=20$; Fig. II.S2*A* and *B* and Table II.S4). After extensive behavioural experiments on dummies (frozen minors) we were able to rule out hindgut content and the Dufour's and poison gland reservoirs as triggers of the behaviour. We ultimately identified the mandibular gland reservoir as the most likely candidate. We further support this hypothesis by applying the gland content on healthy individuals, which then were carried back by their nestmates (Fig. II.5*C* and Table II.S4). A gas chromatographic/mass spectrometry analysis identified dimethyl disulphide (DMDS) and dimethyl trisulphide (DMTS) as the main components within the gland, confirming a previous analysis of the gland contents and concentrations (14ng of DMDS and 5ng of DMTS per gland) (Longhurst et al. 1979). While 9ng of DMDS alone was not enough to trigger the rescue behaviour on a dummy, 9ng of DMTS by itself sufficed. A more pronounced response was achieved with an equal mixture of the two components (9ng of DMDS and DMTS each) (Fig. II.5*D* and Table II.S4).

Value of rescue behaviour for the colony

The rescue behaviour in *M. analis* reduces the foraging costs through a reduced mortality risk. We provide a simple analytical model (additional information in Materials and Methods section: rescue behaviour model and quantification of model) that identifies critical factors promoting the evolution of such rescue behaviour and why it may have evolved so rarely.

We consider that such a behaviour could only emerge in species that forage or hunt in groups and in a limited spatial domain so that injured individuals are likely detected by other nestmates. Our model identifies three additional key variables that affect the potential benefit of such a rescue behaviour: (i) The product of the absolute rate at which ants are severely injured (or killed) in conflict with termites ε_H and the fraction f ($0 \leq f \leq 1$) of these ants that could profit from the rescue behaviour. (ii) The baseline mortality μ_0 of ants – helping is more profitable if μ_0 is small compared to ε_H. (iii) The future added mortality rate μ_J of individuals that were injured and rescued.

The foraging behaviour of *M. analis* seems to offer ideal conditions for rescue behaviour to arise. Injury rates in combat (ε_H = 0.17% per day, for detailed calculations see Supplemental Experimental Procedures) seem to be large compared to the general mortality rate (μ_0 = 0.76% per day) but injuries are rarely fatal (6 observation of fatal injuries in 53 raids). Further, ants that recently lost a leg or had termites clinging onto extremities are significantly hindered in their movement. This presumably makes returning to the nest on their own costly in terms of energy and time needed, thereby prolonging exposure to potential predators and signalling a vulnerable state. These effects result in a high mortality risk for injured individuals if not helped of 32% (Fig. II.2*A*). Carried injured individuals therefore benefit greatly from the rescue behaviour, by reducing that risk to close to 0% (injured ants that were marked and rescued were observed again in subsequent raids in 38/40 cases and were never observed to die during the rescue process). Injured ants carried back recover from injuries in a short time, i.e. parameter μ_J is close to ε_H, if we conservatively assume a second injury to be fatal. The fact that 21% of all ants carry some type of long-term injury in the raiding column (Fig. II.4) indeed substantiates the great value of helping injured nestmates; a conservative estimate as non-permanent injuries are not included in this estimate. This is reflected in the ultimately calculated sustainable colony size in our model, which amounts to a 28.7% larger colony size compared to colonies without the behaviour.

DISCUSSION

This study shows the adaptive value of rescue behaviour in a social predator specialized on a highly defensive prey. A behaviour specifically focused on rescuing injured and handicapped individuals, remarkably also individuals that have permanent injuries in the form of lost extremities. Furthermore, by showing that this behaviour is induced by pheromones, we support the hypotheses that the convergent evolution of rescue behaviour in different taxa has led to distinctive triggering mechanisms, like chemical communication in insects or empathy in humans and possibly other mammals (Bartal et al. 2011, Decety et al. 2016), but see (Vasconcelos et al. 2012) for other interpretations.

Rescuing injured individuals

Intermediates and minors carry injuries considerably more often than majors (Fig. II.4). The division of labour at the hunting ground could explain this discrepancy. While the smaller ants enter the termite galleries to hunt termites, the majors mostly focus on breaking up the protective soil layer over the hunting ground and carrying back the dead termites (Villet 1990). Minors and intermediates are therefore far more exposed to injury risks.

A considerable amount of ants that were carried did not seem to be injured (Fig. II.1). Either the injuries were too small to be detected by the naked eye or these ants were truly unharmed. Most of the ants are picked up at the hunting ground after the fight when the ants are preparing to leave. One possibility could be that the majors running over the hunting ground searching for left over termites or injured individuals are less selective in what to carry. If they still encounter a minor, which might have lagged behind due to it being inside the termite galleries, the major might just pick it up, thereby preventing it from falling behind even further while the column is already leaving.

The fact that experimentally injured ants are not picked up during the outward journey towards the termites seems to suggest that the behaviour is context specific (Fig. II.5*B*). It seems unlikely for the ants to ever encounter this situation naturally. Furthermore the rescue behaviour would have to deviate from the natural one. If the helping ant would carry the injured individual back to the nest, it would expose itself to considerable predation risks by being forced to return alone, while the rest of the column keeps marching to the termites. The other possibility would be to carry the injured ant all the way to the hunting ground only to have to carry it back to the nest afterwards. The different response necessary by the helper ant in this situation and the very low injury risk on the outward journey most likely prevented the ants from developing a corresponding response.

We were able to show that this behaviour is triggered through the chemical compounds DMDS and DMTS harboured in the mandibular gland reservoir. Thereby discovering for the first time a pheromone induced rescue behaviour that seems to be specifically released when the individual is injured. The only other known species harbouring this pheromone is the solitary hunting ponerine ant *Paltothyreus tarsatus*, in which it triggers digging behaviour, most likely to rescue trapped nestmates (Crewe & Fletcher 1974). This species is in the same genus group as *Megaponera* (Schmidt & Shattuck 2014), but being a solitary forager probably has not evolved the same kind of rescue behaviour.

Cooperative self defence has also been observed in *M. analis*, a behaviour during which nestmates scanned each other's legs and antennae and removed *Dorylus* sp. (driver ants) clinging to their extremities (Beck & Kunz 2007). The removal of these *Dorylus* ants seems to follow a similar mechanism to the removal of termite soldiers within the nest.

Evolution of helping the injured

We were able to assess the value of this behaviour for the injured individual, since this is the main benefit of the rescue behaviour it is paramount for understanding the evolutionary benefit of the behaviour for the colony. Ants that had lost an extremity do not immediately switch to four or five legged locomotion mechanisms but keep tripping over their phantom limbs. Ants that had termites clinging on them were even more severely handicapped in their movement (Fig. II.1*B*). These ants were therefore unable to keep up with the returning column, fell behind and thus became isolated from their nestmates. This, on top of their reduced dexterity, increased their predation risk considerably (Fig. II.2*A-E*). Once termites clinging on to their extremities were removed within the nest, they were able to fully perform again in future raids without any clear handicap. Ants that had lost an extremity had the benefit, after being carried back, to recover from their injury in the safe environment of the nest, allowing them to get accustomed to a 4 or 5 legged locomotion. They thus reached running speeds similar to that of uninjured ants 24 hours later (Fig. II.3). Since nearly all injured ants were observed in subsequent raids we conclude that they carried no obvious long-term handicaps from their injuries and may fully participate again in colony tasks.

This type of rescue behaviour, focused specifically on injured and handicapped individuals after hunting, is unique in social insects. Although the benefits seem obvious, there are several reasons as to why this has not yet been discovered in other species. First, because this behaviour can only evolve in group-hunting species, where an injured ant can be detected by their nestmates, it excludes all solitary hunting species as potential candidates. Second, it is also essential that hunting occurs in isolated events, thus creating the risk for the injured ant to be separated from the group during the return journey. In a constantly occupied trail between ants and food source the increased risk carried by an injured ant would be marginal, since it is constantly surrounded by nestmates warding off potential predators. In *M. analis* the outward and return travel are conducted as an isolated column with all ants marching together. The fact that the ants wait after the fight so that all ants may gather before returning to the nest (Bayliss & Fielding 2002, Corbara & Dejean 2000) exemplifies the importance of returning as a group. Thirdly, the predated species must be able to inflict a high amount of non-lethal injuries from which the ants are able to recover. Termites, with their large soldiers, fulfil this criterion as prey. Many group foraging ant species, which focus on leaf-cutting, nectar, seeds or scavenging are thus less likely to develop such a rescue behaviour. Fourthly, the benefit to the colony by the rescued ant has to outweigh the cost of help. In *M. analis* the majors carry back termites and injured individuals, since mostly minors attain injuries (Fig. II.4), the additional task for the majors to carry them back seem minimal from an energetic point of view (Fig. II.S1*A*). Moreover, since on an average raid, only 30% of the ants carry back prey (Bayliss & Fielding 2002), there is a large part of the workforce that is available to help the injured individuals without decreasing the profits of the raid. As the cost of helping an injured ant is therefore likely to be marginal in *M. analis* it is thus ignored in our model. Lastly, the value of an individual for the colony plays an important role. This can be approximately quantified through the mean mortality rate in a colony. For a colony to be in equilibrium the number of ants being born has to match the mortality rate and in equilibrium the population turnover is directly related to the lifespan of the individual. In *M. analis* the population turnover is relatively low, with a birth rate of only 13 ants per day, demonstrating again the importance of rescuing the injured. Species with a very high turnover, like army ants, would therefore benefit less from saving one injured ant, although this still remains to be tested. The specific biology of *M. analis* therefore provides the right circumstances where the benefit of saving the injured is especially large. We thus argue that this behaviour evolved as part of an evolutionary arms race against termites, as a mean of minimizing losses during raids and therefore foraging costs.

Rescue behaviour has been previously observed in ants (Hollis & Nowbahari 2013a), but in very different contexts. Excavating trapped nestmates after a cave in or rescuing an ant that fell in an antlion trap are both situations in which the individuals are confronted with an imminent danger, i.e. suffocation or being eaten (Hangartner 1969, Hollis & Nowbahari 2013a, Hollis & Nowbahari 2013b). This is not the case in our situation, not only are the injured ants in many cases handicapped for life through the loss of extremities but the danger towards these ants is far less obvious. There is no direct threat to the injured ant but rather an abstract increased predation risk if these ants were to return alone. This demonstrates that complex rescue behaviour can evolve in very unique situations if the necessary drivers are present, even in species that are very likely unable to recognize the increased risks to which they are exposed to.

OUTLOOK

Our observations offer a unique opportunity to experimentally study the evolutionary drivers leading to the emergence of rescue behaviour in animals: Injury and predation rates can be manipulated, rescue behaviour can easily be prevented, and critical variables and parameters can be measured. *Megaponera analis* Pan-African distribution should also allow us to study the degree of fine-tuned adaptations to differing external selection pressures prevailing in different ecosystems. This would allow us to identify the potential driving factors most important for this behaviour to arise. The model also helps us to identify other potential species where this behaviour might be found. There are other ponerine genera, like *Leptogenys*, which focus on hunting termites with some of them hunting in groups (Ganeshaiah & Veena 1991), examining their raiding behaviour in more detail could be promising. Slave making ants could potentially also fulfil the criteria, if their prey is able to inflict a significant amount of non-lethal injuries.

SUPPLEMENTARY MATERIAL II

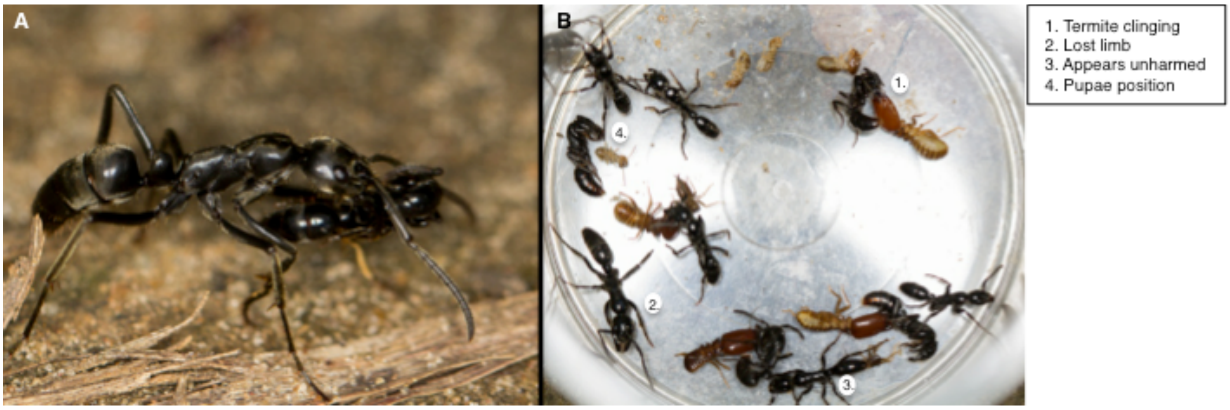

Fig. IIS1. Illustration of a helping ant and different injury types as shown in Fig. II.1. (A) Injured minor being carried by a major nestmate. **(B)** Different types of injury after a raid, with 1. A dead termite soldier clinging on to a minor (termite clinging); 2. A major having lost its left hind leg (lost limb); 3. An intermediate appearing unharmed to the naked eye (appears unharmed) and 4. An injured minor assuming the pupae position after being antennated by a nestmate (pupae position).

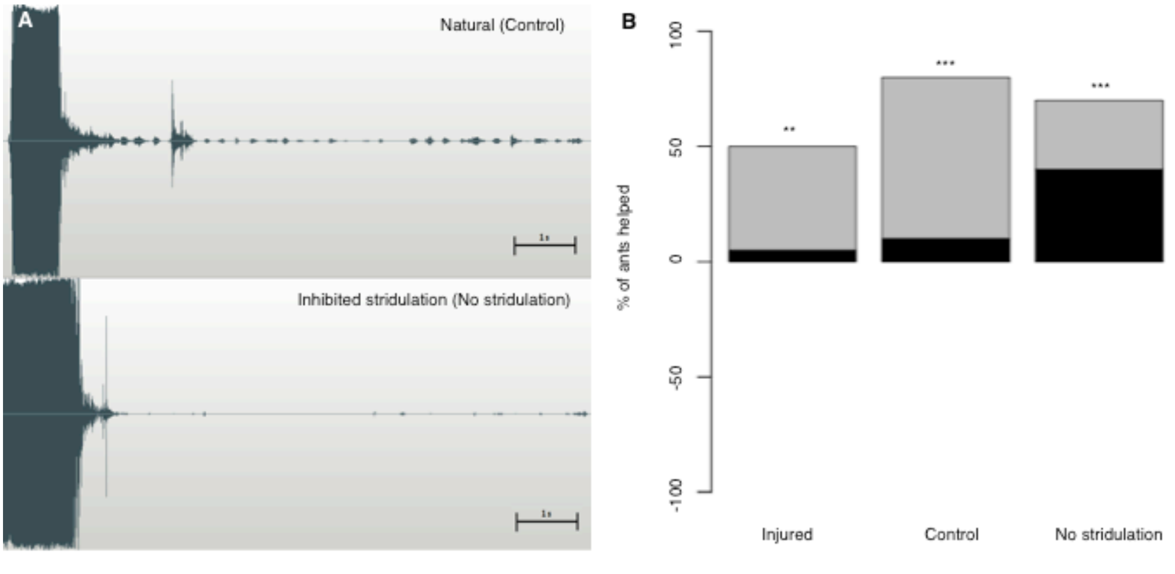

Fig. II.S2. Effect of stridulation on rescue behaviour. (A) Sonogram before (top) and after (bottom) disabling the stridulatory organ of *M. analis*. **(B)** Behavioural response of helper ants towards manipulated ants (*N*=20). Injured: artificially injured ant, cutting off two legs; Control: artificially injured with acrylic colour on thorax; No stridulation: artificially injured with acrylic colour covering the stridulatory organ on the gaster.

Table II.S1. Statistical differences in injury type frequency (Fig. II.1*A*) and speed (Fig. II.1*B*) in injured *M. analis* ants (Kruskal-Wallis test followed by pairwise comparisons with Bonferroni-corrected Dunn's test; *N*=20 per test). Abbreviations: Healthy: Control with healthy ant. Lost limb: ant that partially or completely lost an extremity; Termite clinging: ant that has a termite clinging to its body; Carried unharmed: ant with no discernible injury.

Injury type 1	**Injury type 2**	***Z***	***P***
(A) Kruskal-Wallis test			<0.001***
Lost limb	Termite clinging	-2.6	0.014*
Lost limb	Carried unharmed	0.12	1
Termite bite	Carried unharmed	2.72	0.009**
(B) Kruskal-Wallis test			<0.001***
Healthy	Lost limb	2.89	0.01**
Healthy	Termite clinging	6.29	<0.001***
Healthy	Carried unharmed	1.62	0.3
Lost limb	Termite clinging	3.40	0.002**
Lost limb	Carried unharmed	-1.27	0.61
Termite clinging	Carried unharmed	-4.67	<0.001***

Table II.S2. Statistical differences in running speed of individuals with different stages of injury as shown in Fig. II.2 (Kruskal-Wallis test followed by Dunn's test with Bonferroni-correction; *N*=20 per test). Abbreviations: Fresh = Ant that freshly lost a leg on each side; Old = Same ant 24 hours later; Healthy = Control with an uninjured ant.

State 1	**State 2**	***Z***	***P***
Kruskal-Wallis test			<0.001***
Fresh	Old	-3.07	0.003**
Fresh	Healthy	3.86	<0.001***
Old	Healthy	0.78	0.65

Table II.S3. Statistical differences in long term injuries in the different castes as shown in Fig. II.4 (ANOVA test followed by Tukey HSD test; *N*=20 per test).

Caste 1	**Caste 2**	**lwr**	**upr**	***P***
ANOVA				<0.001***
Major	Intermediate	6.14	22.86	<0.001***
Major	Minor	10.5	27.26	<0.001***
Minor	Intermediate	-3.96	12.76	0.4

Table II.S4. Statistical differences in significance of rescue behaviour compared to behaviour of healthy individuals as shown in Fig. II.4 *A-D* and Fig. II.S2 (Fisher's exact tests for count data between treatment healthy (no help) and the other categories with Bonferroni correction). Abbreviations: DMDS = Dimethyl disulphide; DMTS = Dimethyl trisulphide; DMDS/DMTS = 50/50 Solution of dimethyl disulphide and dimethyl trisulphide in hexane; Mg dead = Mandibular gland on dead dummy; Mg alive: Mandibular gland on living nestmate. For explanation of treatments see Fig. II.5 *A-D* description.

Treatment	***P***	***N***
Healthy	1	20
Dead	1	20
Foreign	<0.001***	20
Way out	1	20
Hunting	<0.001***	20
Return	0.0013**	20
No stridulation	<0.001***	20
Colour control	<0.001***	20
Mg dead	<0.001***	20
Mg alive	0.0013**	20
Dufours	0.013*	20
Poison	0.2	20
DMDS	0.19	20
DMTS	<0.001***	20
DMDS/DMTS	<0.001***	20
Hexane	0.9	20

Dead *M. analis* major scavenged by *Pheidole* sp. workers

CHAPTER III: WOUND TREATMENT AND SELECTIVE HELP

Submitted as: Frank ET, Wehrhahn M, Linsenmair KE (submitted) Saving the injured: wound treatment and selective help in the ant *Megaponera analis*. *Proceedings of the Royal Society B*

ABSTRACT

Open wounds are a major health risk in animals, with species prone to injuries likely developing means to reduce these risks. We therefore analysed the behavioural response towards open wounds on the social and individual level in the termite group-hunting ant *Megaponera analis*.

During termite raids some ants get injured by termite soldiers (in the form of cut extremities), after the fight injured ants get carried back to the nest by nestmates. We observed treatment of the injury by nestmates inside the nest through intense allogrooming directly at the wound. Lack of treatment increased mortality from 10% to 80% within 24 hours, most likely due to infections. Wound clotting occurred extraordinarily fast in untreated individuals, within ten minutes. Furthermore, heavily injured ants (loss of five extremities) were not rescued or treated; this was regulated not by the helper but by the unresponsiveness to cues by the injured ant. Interestingly, lightly injured ants behaved "more injured" near nestmates.

We show organized social wound treatment in insects through a multifaceted help system focused on injured individuals. This was not only limited to selective rescuing of lightly injured individuals by carrying them back (thus reducing predation risk), but moreover includes a differentiated treatment inside the nest.

INTRODUCTION

Open wounds are a major mortality risk in animals (Siva-Jothy et al. 2005) and likely to get infected without treatment. We therefore expect species that are prone to loosing extremities to develop means to reduce the mortality risks these injuries pose. Social predatory species that hunt prey capable of inflicting injuries fit this criterion. Ants generally are assumed to have large colonies in which the individual worker hardly counts (i.e. a very large population turnover: large colony size and high birth rate) (Hölldobler & Wilson 1990). The benefit from helping injured ants in this scenario is small, since replacing them should be easier (Frank et al. 2017). At the same time, if injuries were mainly fatal the benefit of a rescue behaviour focused on injured individuals would again be marginal (Frank et al. 2017). The ponerine group-hunting termite specialist *Megaponera analis* fits all the criteria were a rescue behaviour focused on injured ants has a large benefit for the colony (Frank et al. 2017).

Megaponera analis is found in sub-Saharan Africa (Schmidt & Shattuck 2014) and specialized on hunting termites solely from the subfamily Macrotermitinae (Yusuf et al. 2014, Longhurst et al. 1978, Levieux 1966). These ants leave in groups of 200 to 600 individuals to termite foraging sites, which can be up to 50 meters away, in a column formation led by a scout that previously investigated the foraging site (Yusuf et al. 2014, Bayliss & Fielding 2002, Hölldobler et al. 1994, Frank & Linsenmair 2017a). At the hunting ground division of labour occurs: while the majors break open the soil layer covering the termites, the minors rush into these openings to kill and carry out the prey (Villet 1990, Crewe et al. 1984, Frank & Linsenmair 2017b). The hunting process lasts five to ten minutes after which the termites get collected in the mandibles of the majors and the group returns together back to the nest in the same column formation (Frank & Linsenmair 2017a, Frank & Linsenmair 2017b). During the hunt some ants get injured by termite soldiers, which have strongly sclerotized heads and mandibles (Prestwich 1984). These ants often loose limbs or have termites clinging to them (Frank et al. 2017, Yusuf et al. 2014, Burgeon 1929). Before returning to the nest, nestmates search for these handicapped ants, which call for help with pheromones in the mandibular gland, consisting of dimethyl disulphide (DMDS) and dimethyl trisulfide (DMTS) (Frank et al. 2017). After a short investigation a nestmate picks up the injured ant and carries her back to the nest within the safety of the returning group. However ants that were fatally injured were left behind (Frank et al. 2017). If the injured ants were to return alone to the nest they would die in 32% of the cases during the return journey (Frank et al. 2017). Within the nest the termite soldiers get removed by nestmates, thus fully rehabilitating the handicapped ant. Ants that lost extremities are capable of changing their locomotion to a four or five-legged gait in less than 24 hours and are capable of reaching running speeds similar to healthy ants again (Frank et al. 2017). These injuries occur regularly, with roughly a third of the minors participating in raids having lost a leg at one point in their life (Frank et al. 2017). Saving the injured ants therefore significantly increases the fitness of the colony (Frank et al. 2017). While the benefit of being carried back to the nest is clear (reduced predation risk) it is still unclear what risk open wounds (cut limbs) pose for the injured individual and the colony.

Social insects are especially prone to infections due to the low genetic diversity within a colony and the frequent contacts between individuals, thus facilitating transmission (Cremer et al. 2007). Positive social interactions – e.g. preventing the spreading of an infection through adaptive behaviour – may more than compensate the system beyond the single individual immune competence: social immunity (Cremer et al. 2007, Meunier 2015). This can range from purely prophylactic behaviours like removing corpses and waste from the nest (Howard & Tschinkel 1976), using antimicrobial substances as nest material (Chouvenc et al. 2013) or actively grooming nestmates to keep their cuticles free from parasites (Oi & Pereira 1993). One of the main chemical defences against infections in ants are the secretions of the metapleural and venom gland (Fernandez et al. 2006, Tragust 2016). These glands excrete antimicrobial substances, which during allogrooming by nestmates get spread over the cuticle and thus inhibit infections (Fernandez et al. 2006, Tragust 2016, Tranter et al. 2015). While individuals that suffer from parasites receive more (or depending on infectiousness less) attention from nestmates (Theis et al. 2015), it is still unknown how ants behave towards nestmates with open wounds, like cut off extremities.

We therefore investigated the health risks these open wounds represented for the injured ant and if the ants had developed mechanisms to decrease these risks, both on the individual and social level. Furthermore, while the benefit for the colony of leaving behind fatally injured ants is clear, the mechanism that regulates this behaviour remains unknown: is the decision to rescue made by the helper or the fatally injured ant?

MATERIALS AND METHODS

Experimental design

The study was conducted in a humid savannah woodland located in the Comoé National Park (Konaté & Kampmann 2010), northern Côte d'Ivoire (Ivory Coast), at the Comoé National Park Research Station (8°46'N, 3°47'W). Experiments and observations in the field were carried out from January to March and July to November 2015, March to April 2016 and April to July 2017 from 7:00-11:00 and 15:00-18:00 (when raiding activity was high (Frank & Linsenmair 2017a)). *Megaponera analis* is found throughout sub-Saharan Africa from 25°S to 12°N (Schmidt & Shattuck 2014). We observed 208 raids of 16 different colonies of *M. analis* on which the predominantly hunted termite genus was *Pseudocanthotermes*. Colony size for 14 excavated colonies was between 900-2300 ants, a result comparable to previous studies in other regions (Villet 1990, Yusuf et al. 2013). Even though *M. analis* is known to show monophasic allometry within its worker sizes (Villet 1990, Crewe et al. 1984) for statistical analysis and illustration, the workers were divided into majors (head width > than 2.40 mm), minors (head width < 1.99 mm) and intermediates (head width 2.40 - 1.99 mm) as proposed by Villet (1990).

Ethogram of selective help

Previous studies showed no significant difference in the number of ants helped at the hunting ground or on the return journey (Frank et al. 2017). We therefore conducted experiments with heavily injured ants during return journeys for easier reproduction of trials. The experiments were each repeated 20 times with at least 5 different colonies per experiment, each returning raid was only used for one trial. An injured ant (or a dummy: frozen dead ant coated with the synthesized help pheromone, consisting of a 50/50 solution of DMDS and DMTS) was placed at the front of the return column at least 1 m away from the hunting ground. All behavioural reactions by the nestmates were recorded until the whole column had passed the study subject. The behavioural reactions of the helping ants consisted of five categories: 1. Ignored: Contact with the study subject was less than 2 seconds; 2. Investigated: The study subject was antennated for more than 2 seconds; 3. Picked up: The study subject was fully lifted from the ground; 4. Carried back: The study subject was carried back for at least 20 cm towards the direction of the nest; 5. Carried away: The study subject was removed from the return column in a direction that was not the one back to the nest, i.e. away from the column. For statistical analysis we only identified behaviour 4 (carried back) as a successful rescue behaviour. Data for lightly injured ants (2 legs experimentally removed) and dummy were taken from Frank et al. (2017). Heavily injured ants had 5 legs randomly removed with scissors at the femur. To incapacitate the legs without removal they were dislocated with a pair of forceps. To quantify antennation/investigation time by helpers the time was noted between the first antennation of the first helper on the study subject until antennation by the helper ended (the trials were filmed). The antennation time for the ant that ultimately helped the injured was also quantified.

Visual injury reinforcement

During the return journey of a raid a healthy minor was carefully removed with forceps and had two randomly selected legs removed at the femur. These ants were then either placed at the centre of the returning raid column or on the return pheromone trail one minute after the raid column had passed. The running speed (cm/s) was measured for the next 60 seconds. The same experiment was conducted with healthy ants as control. Each raid was only used for one experiment (n=20 per experiment (4) for n=80 raids).

To see whether injured ants were picked up at the hunting ground or on the return journey, we removed all ants carrying nestmates together with the carried ant from a returning raid column at two points. Once directly after leaving the hunting ground and once directly before arriving at the nest. This was done for a total of 8 raids in 3 different colonies. Raid column speed was calculated by quantifying the time it took the front of the column to move from the hunting ground back to the nest and measuring the distance, this was done for a total of 82 raids.

Laboratory colonies

Fourteen colonies were excavated and placed in artificial nests in the field stations lab (colony size 1293±543 ants). Nests (30x20x10 cm) were made of PVC and connected to a 1x1m feeding arena. The ground was covered with soil from the surrounding area. In the feeding arena *Macrotermes bellicosus* termites were placed, which were collected from the surrounding area by using pots filled with dry grass. These termites were found by scouts and triggered raiding behaviour. For further details on lab keeping see Yusuf et al. (2013).

In six of these colonies all individuals were carefully examined for any lost extremities so as to quantify the percentage of injured ants in a colony (in total 7240 ants were analysed).

Ethogram of nest treatment

Ants were experimentally manipulated in four different ways in the laboratory. Lightly injured (removal of two legs), heavily injured (removal of five legs), termite bite (major *Pseudocanthotermes* sp. soldier encouraged to bite and cling on to either a leg or thorax) and healthy (control). All were marked with acrylic colour for individual recognition and filmed for the first 3 hours inside the laboratory nests. All manipulated ants were placed in front of the nest entrance directly after a raid finished. They were removed again before the next trial would be conducted. The trials were filmed using a 2 MP IR Bullet IP Camera (ALONMA GmbH) and analysed using VLC media player v.2.1.4 Rincewind (intel 64bit) and the add-on Zoomit v4.4. Observed behaviour was classified into five categories: (1) anntenating: a nestmate touches the marked ant with its antenna; (2) licking: a nestmate licks the open wound; (3) allogrooming: the subject gets cleaned by nestmates; (4) pulling: nestmates pulling on the clinging termite and (5) termite: other actions towards the clinging termite, like biting. These five behaviours were quantified for the first 3 hours in 30 min intervals. If the ant was unobservable during the experiment for more than 30% of the time (for example when the subject left the nest) the trial was disregarded completely. This was the case for 5 out of 15 trials with termites clinging on ants, for 16 out of 26 trials with lightly injured ants, for 8 out of 17 trials with heavily injured ants and for 9 out of 15 trials with healthy ants.

Isolation trials

For the isolation trials we removed two randomly selected legs at the femur with sterilized scissors. All individuals were taken from laboratory colonies on the return journey of a raid (*N*=6 colonies). For each experiment 20 ants (*N*=20) were then separately placed inside cylindrical glass containers with a diameter of 3 cm and a height of 5 cm. This container was filled with soil from the surrounding area up to a height of 1 cm. To create nest like humidity conditions the soil was moistened with 1 ml of water and covered with aluminium foil. For the sterilization trials the container (together with the soil) was placed for 3 hours at 220 °C in an oven together with the forceps and scissors. The water used for the trials was boiled for at least 10 minutes. The injured ant was then placed in the container and observed for the next 24 hours, if no reaction was observed even after shaking the container the ant was classified as dead.

To test for possible influence/treatment of nestmate behaviour in the nest, injured ants were placed outside the entrance of a laboratory colony after a raid directly after inflicting the injury. The ant was marked with acrylic colour for individual recognition and removed from inside the nest either after 1 or 12 hours to be placed in the isolation container for the subsequent 24 hours.

Statistical analysis

For statistical analysis and graphical illustration 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). We tested for deviations from the normal distribution with the Shapiro Wilks test ($P>0.05$). A Bartlett test was used to verify homoscedasticity ($P>0.05$). Since this was not the case for all our data a Kruskal-Wallis rank sum test was used, followed by a Dunn's test with Holm-Bonferroni correction. To analyse the ethogram data a Fisher's exact test with Bonferroni correction was used with a no help control (0 out of 20 helped) compared to our treatments. To test for significant differences in mortality of the isolation trials we conducted a Cox proportional hazards regression model. Median values mentioned in the text are followed by a median absolute deviation. Box plots show median (horizontal line), interquartile range (box), distance from upper and lower quartiles times 1.5 interquartile range (whiskers) and outliers (dots) > 1.5X upper or lower quartile.

RESULTS

Nest Treatment

Handicapped ants were antennated far more often than healthy control ants, especially during the first hour after injury (Fig. III.1*A* and Table III.S1). Ants that had lost two limbs were frequently licked directly at the injury within the first hour (Fig. III.1*B* and Table III.S2). The remaining part of the cut limb was always held upwards and nestmates carefully held the injured limb in place with their mandibles and front legs, this allowed them to intensely lick directly into the wound for up to four minutes at a time (Fig. III.S1*AB*). Ants that had termites clinging to them had nestmates pulling on the termite, with the handicapped ant pulling in the opposite direction (Fig. III.1*C* and Table III.S3).

We also observed ants biting the clinging termite, specifically on the area of the pronotum connected to the head. After some time this behaviour led to the removal of the termite body, with the head remaining in place (Fig. III.S1*C*). In 3 out of 10 cases the termite was removed successfully within 30 to 60 min and in two further cases the termite was removed in less than 24 hours (in the other five trials the termite was not removed within the first 24 hours). In one case the termite head remained clinging on the ant even two weeks later (while the termite body was removed).

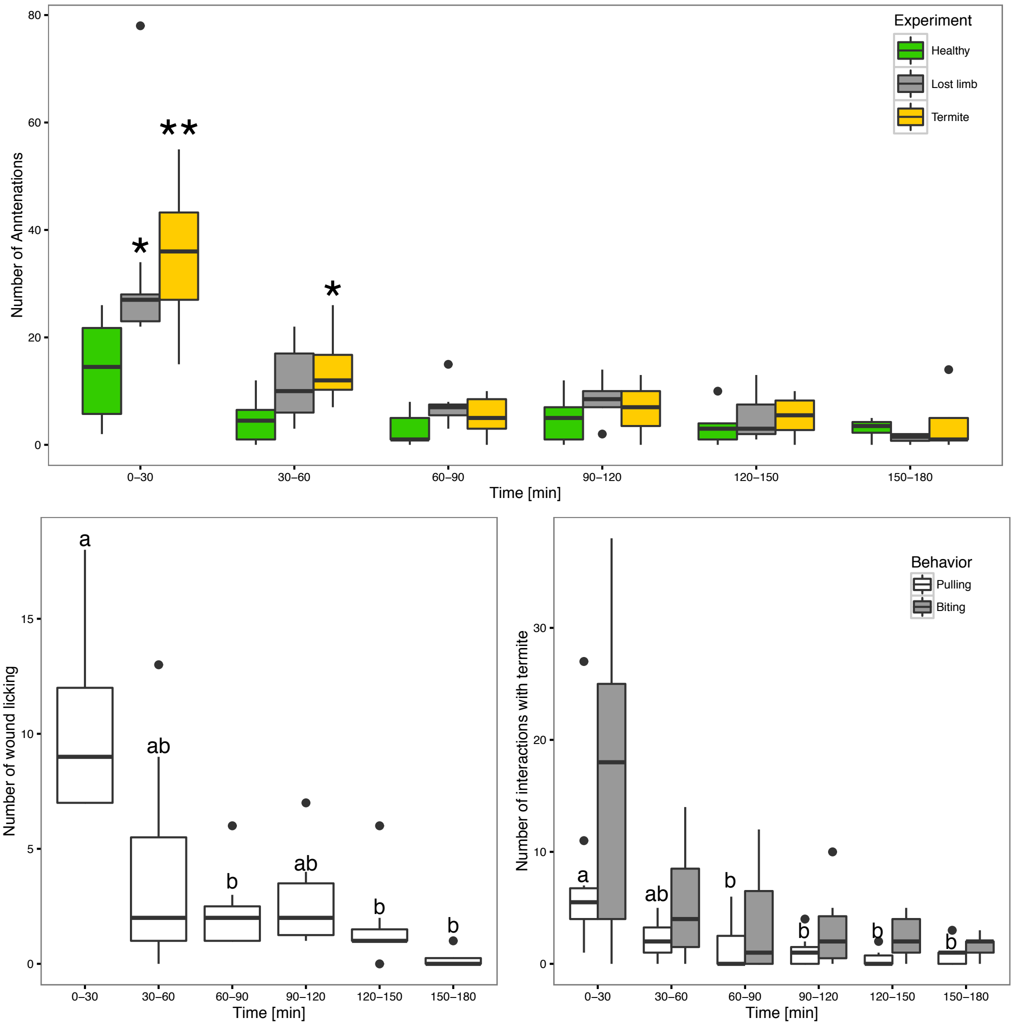

Fig. III.1. Treatment of handicapped and injured ants inside the nest. (A) Number of antennations by nestmates for differently treated individuals inside the nest in 30-minute blocks. Lost limb: injured ants with two cut off limbs; Termite: ant with dead termite soldier clinging on it; Healthy: control experiment with healthy ant. Sample size for lost limb: *N*= 10; termite: *N*=10; Control: *N*=6. **(B)** Number of times wound licking by nestmates on injured ants (two cut off limbs) was observed; *N*=10. **(C)** Number of times interactions with the clinging termite by nestmates was observed. Pulling: nestmates were pulling on the termite. Biting: nestmates were biting the termite (no significant difference); *N*=10. Statistical analysis was always a Kruskal-Wallis rank sum test followed by a Dunn’s test with Holm-Bonferroni correction. See also Table III.S1–3 for detailed statistical results.

The majority of allogrooming by nestmates was concentrated on the acrylic colour marking on the ant, the number of these interactions remained relatively constant throughout the 3 hours of observation, with a small peak in the first 30 min (Fig. III.S2 and Table III.S4). Nestmates were observed to carry heavily injured ants out of the nest within the first 30 min of the trial and since the heavily injured ants did not return into the nest this led to the termination of all trials (*N*=9). In the first 30 minutes heavily injured ants were licked directly at the wound significantly less often than lightly injured ants (Wilcox test: *W*=3, *P*<0.001). Due to the constant removal of heavily injured ants from the nest they were excluded from the overall analysis, but see Fig. III.S3 for the ethogram of heavily injured ants for the first 30 min with comparison to the other groups. Heavily injured ants were always found dead in the foraging arena within the subsequent 24 hours.

Survival rate

To test for possible benefits of the treatment on lightly injured ants we isolated minors that had two extremities cut off under nest-like conditions. On unsterile soil the injured ants had a mortality of 80% within the first 24 hours (*N*=20; Fig. III.2 and Table III.S5), while the mortality was only 10% when the injured ants had received a one hour treatment beforehand by their nestmates (*N*=20; Fig. III.2). To test if this treatment inhibited infection of the wound we isolated injured minors in a sterile environment: this led to a mortality of only 20% in 24 hours (*N*=20; Fig. III.2). Furthermore, a freshly cut wound appeared to be completely sealed/clotted within ten minutes, without interaction by nestmates (Fig. III.S4).

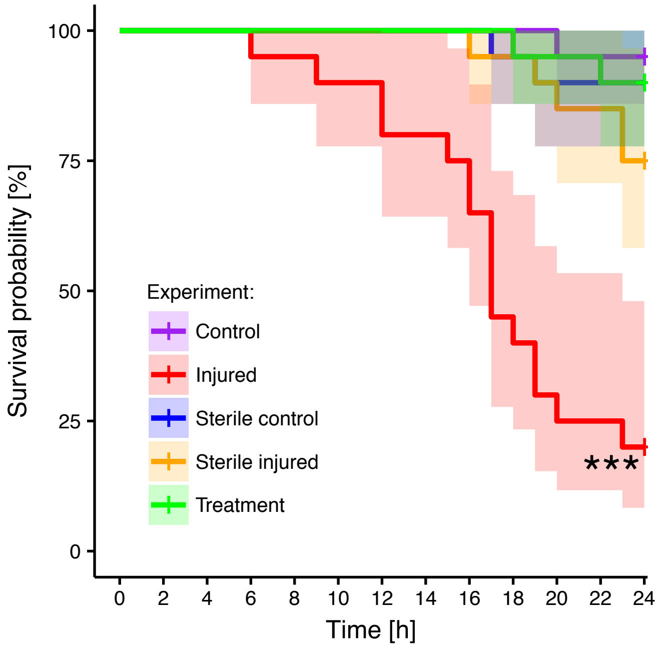

Fig. III.2. Survival probability of differently treated ants. Kaplan-Meier cumulative survival rates of workers in isolation that received different treatments. Shaded area represents standard deviation. Control: healthy ant kept on unsterile earth; Sterile control: healthy ant kept on sterile earth; Injured: ant with two removed limbs kept on unsterile earth; Sterile injured: ant with two removed limbs kept on sterile earth; Treatment: ant with two removed limbs kept in the nest for 1 hour before being isolated on unsterile earth. N=20 for all treatments. ***:P<0.001. Statistical significance tested with a Cox proportional hazards regression model (Table III.S5).

Selective help. In the six analysed colonies we found that significantly more ants had lost one limb (4.2 ± 1.1 %; n=292 injured) than two (0.7 ± 0.2%; N=46 injured) or three limbs (0.2 ± 0.1%; N=17 injured) and none were more severely injured (Kruskal-Wallis test: X^2_2=39.3,P<0.001; Dunn test: once vs twice: Z=4.1, P<0.001; once vs thrice: Z=6.2, P<0.001; twice vs thrice: Z=2.1, P=0.02). Minors and intermediates made up the majority of injured ants (Fig. III.S5).

We experimentally tested if rescue behaviour was only concentrated towards lightly injured ants (two lost extremities) or also towards heavily injured ants (five lost extremities). While lightly injured ants were carried in 45% of the cases on the return journey (N=20), we only observed rescue behaviour in one case on a heavily injured ant (5%, N=20; Fig. III.3*A*). Interestingly nestmates investigated heavily injured ants significantly longer than lightly injured ants (Fig. III.3*B*). To rule out potential leg counting as the selective force we incapacitated 5 legs with forceps without removing them. While this led to more nestmates trying to pick up the injured ant, they were rarely carried back to the nest (Fig. III.3*A*). Applying the synthesized help pheromone DMDS and DMTS on a heavily injured ant significantly increased the number of pick up attempts and carried ants

(Fig. III.3*A* and Table III.S6). The injured ant kept flailing around, turning on its axis and ignoring their nestmates, making it considerably harder for the nestmates to pick up the injured ant and leading to longer investigation times (Fig III.3*B* and Table III.S7).

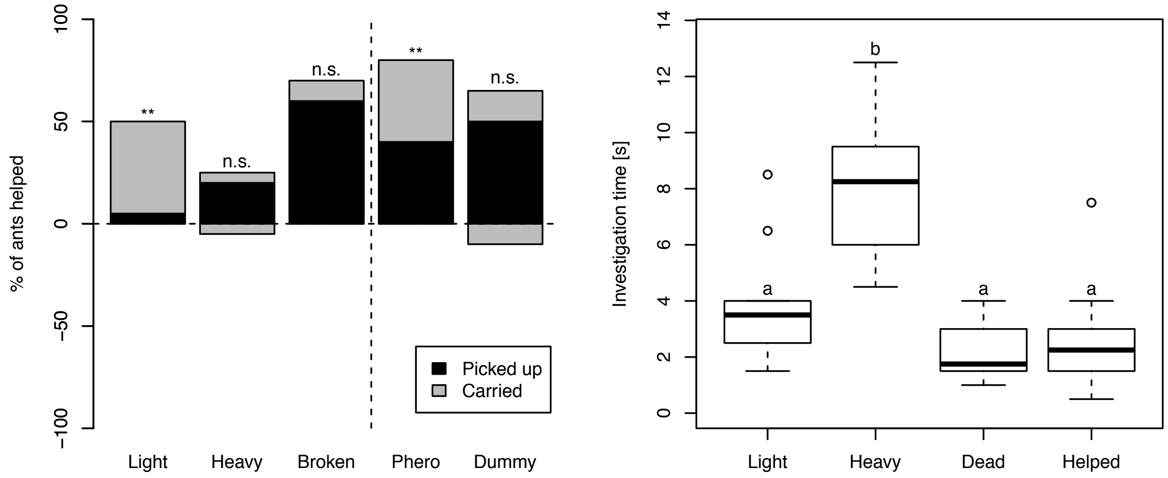

Fig. III.3. Injury dependent help. (A) Rescue behaviour in *M. analis* to differently injured individuals. Light: lightly injured individual (two cut off legs); Heavy: heavily injured individual (five cut off legs); Broken: Ant with incapacitated legs; Phero: heavy injured ant coated with synthesized help pheromone (DMTS/DMTS); Dummy: frozen dead ant coated with synthesized help pheromone. Positive values show clear attempts of help by picking up the ant and dropping it again (black) or carrying it back to the nest (grey). Negative values show behaviour in which the ant was disposed of (dragged away from the raiding column). Fisher's exact test for count data between neutral treatment (zero help) and the other categories for carried ants (see Table S6 for detailed statistical results); *N*=20. Data for light and dummy trials from Frank et al. 2017. **(B)** Investigation time by nestmates on injured individual. Dead: frozen dead ant; Helped: Time of investigation for ants that were helped. Kruskall-Wallis rank sum test followed by a Dunn's test with Holm-Bonferroni correction; *N*=20. See also Table III.S7 for detailed statistical results.

Visual reinforcement of injury

Even though all injuries are inflicted at the hunting ground only 61% of carried ants were picked up there. The rest was picked up during the return journey (*N*=8 raids with 38 carried ants). Ants that had a termite clinging to them were almost always picked up at the hunting ground (94 ± 18%; *N*=16 ants with clinging termites). Ants that lost a limb or appeared unharmed were mostly picked up during the return journey (Picked up at hunting ground: lost limb: 27±29%, *N*=13; carried unharmed: 13±23%, *N*=9).

We noticed that injured ants (two lost limbs) behaved markedly different to healthy ants when placed at the front of a returning raid column. While healthy ants resumed the speed of the column, injured ants moved significantly slower and kept falling over. This was in strong contrast to the speed achieved both by healthy and injured ants when released alone on the return pheromone trail (Fig. III.4 and Table III.S8). This behaviour even changed within the same trial: while an injured ant barely moved forward when nestmates were close, after the returning raid column had passed by without helping her, the injured ant immediately started to follow them.

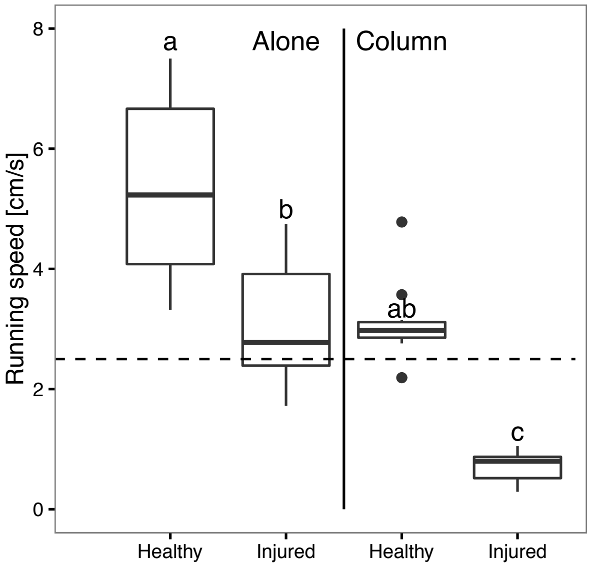

Fig. III.4. Context specific behaviour of injured ant. Running speed of healthy and injured (-2 legs) ants depended on presence/absence of raiding column. Dashed line: mean returning raid-column speed (2.2 cm/s, *N*=82 raids). Kruskal-Wallis rank sum test, followed by a Dunn's test with Holm-Bonferroni correction; *N*=20. See also Table III.S8 for detailed statistical results.

DISCUSSION

This study shows a multifaceted rescue system focused on rehabilitating long-term injured individuals (in the form of lost extremities). This is not only limited to rescuing the injured by carrying them back from the hunting ground, thus decreasing predation risk (Frank et al. 2017), but furthermore includes a differentiated treatment inside the nest, which significantly reduces mortality of the injured. We further show a type of helping "triage", with heavily injured ants not receiving help or treatment, without the necessity of a conscious decision-making process. Lastly we show that injured ants change their behaviour according to the proximity of nestmates.

Nest treatment

We observed wound licking/treatment by nestmates on injured individuals inside the nest. This treatment was mostly confined to the first hour after injury and reduced mortality when compared to isolated untreated ants by 80%. Termite soldiers clinging on to ants were also removed by nestmates through pulling and focused biting on the termites pronotum.

The cuticle is one of the main barriers against pathogens (Siva-Jothy & Rolff 2005). Injuries occur at termite foraging sites (Frank et al. 2017) under very unsterile conditions, it thus seems likely that infections at the wound can occur. This hypothesis is supported by the increased survival chance of injured ants in a sterile environment. The treatment by nestmates was clearly focused on the wound and led to intense licking/allogrooming directly into the open wound (Fig. III.S1), sometimes uninterrupted for several minutes. Since this was the only type of observed interaction we hypothesize that dirt and debris were likely removed and potentially antimicrobial substances were applied, although this remains to be tested.

Medication in the animal kingdom is rare, especially on other individuals. In apes self-medication has been observed, by including medicinal plants in their diet when sick (Huffman 2003). Cognition is not mandatory though for the evolution of medication. Fruit flies and caterpillars are equally capable to ingest toxic substances as a form of medication against parasites (Singer et al. 2009, Milan et al. 2012, de Roode et al. 2013). Monarch butterflies perform a type of trans-generational medication, by placing their eggs on antiparasitic milkweed (Lefevre 2010). In social insects social immunity and cooperation play a crucial role when confronted with parasites (Cremer 2007). Wood ants (*Formica paralugubris*) use antimicrobial resin in their nests as prophylaxis (Castella 2008) and honeybees (*Apis mellifera*) even increase resin collection pro-actively when parasitized (Simone-Finstrom 2012). There are many more examples of colony responses and organization to parasite infections on a colony level (Ugelvig & Cremer 2007, Cremer et al. 2007), our observations are more focused on the level of the individual. It has been previously shown that ants disinfect fungus-exposed brood through allogrooming (Tragust et al. 2013) and that grooming overall leads to parasite reduction on treated individuals (Pritchard 2016, Hughes et al. 2002). Our observations are the first, to our knowledge, to show this type of treatment to be directed towards a high-risk infection zone of an individual (open wounds). While parasite removal on the cuticle of healthy individuals (allogrooming) serves a similar purpose (to prevent parasitation/infection of the treated individual) the marked difference is that in our case the treatment seems to be more prophylactic rather than reactionary. In our observations the treatment occurs directly after the injured ant re-enters the nest, thus making an actual infection unlikely to have broken out after such a short time period (1-5 minutes after injury). Moreover debris and dirt are likely always encountered on the cuticle of ants, the fact that treatment is only focused on the injury shows the context dependent importance for the classification of infection risk agents. On an intact cuticle dirt is a minor infection risk, on an open wound the infection risk is far greater. The fact that wound clotting also seems to occur remarkably fast (within ten minutes) further shows that behaviours to reduce high injury risks are not only on the level of the colony but also has incentivized adaptations on the level of the individual.

This is the first example to show organized social wound treatment in insects, which raises many new questions. How do the ants know where the injury is? How do they know when to stop treating the injury? Is the behaviour purely prophylactic or also therapeutic in case of an infection outbreak? How big is the time-window after injury in which treatment is effective? We hope that further research will help answer these questions.

Selective help

Ants that lost extremities made up 5.1% of the colony, this is in stark contrast to the 21% they make up in the raiding party (Frank et al. 2017). This discrepancy probably has multiple causes. The age polyethism in *M. analis* leads to younger ants being focused mostly on nest tasks (Villet 1990), while older workers go out to forage (i.e. younger ants have a very low injury risk), thus leading to smaller percentages of injured ants within the colony. In addition injured ants might be more motivated to go out and participate in future raids, ants in the species *Myrmica scabrinodis* become more risk prone when injured or poisoned (Moron et al. 2008, Moron et al. 2012), this could also hold true for *M. analis*. Ultimately the high injury discrepancy between raids and the colony as a whole suggests a high work division fidelity.

We observed that heavily injured ants (loss of 5 limbs) were rarely helped by their nestmates. When the help pheromone was applied on the heavily injured ant rescue attempts were more numerous (pick ups) but were rarely successful (Fig. III.3). Our results and observations suggest that cooperation between the rescuer and the injured ant is vital for the pick-up and carry back to the nest to be successful.

Heavily injured ants behave markedly different to lightly injured ants. Lightly injured ants, when antennated by a nestmate, immediately assumed a pupae-like position, which facilitated transportation. This was not the case for heavily injured ants: their legs flailed around constantly and the ant kept turning on its axis, most likely trying to return to a resting position (stand up). Nestmates trying to elicit a reaction by the injured ant had longer investigation times because of it (Fig. III.3*B*) before moving on. To exclude leg counting as a possibility we incapacitated the legs instead of cutting them off, in this case the injured ant was much more immobile (due to the obstacle the stretched out broken legs presented) and was easier to investigate by their nestmates. This led to a much higher pick up rate (Fig. III.3*A*), although carrying was problematic due to the legs not being tucked in, which often led to the helper ant dropping the injured ant again after a short distance. Applying the help pheromone on a heavily injured ant seemed to increase motivation for nestmates to help the ant, but overall the same obstacles were observed. We therefore conclude that rescue behaviour does not occur on heavily injured ants mostly due to the uncooperativeness of the injured ant itself to external cues.

This is further supported by the lack of treatment inside the nest and the heavily injured ant leaving the nest within the first hour or being carried out. This behaviour is very similar to moribund ants leaving the nest when parasitized or close to death (Heinze & Walter 2010, Chapuisat 2010) and has also been previously observed to occur in *M. analis*, with injured ants leaving the nest (Burgeon 1929), although these observations remained unexplained at the time. The uncooperativeness by heavily injured ants at the hunting ground can be compared with results on *Formica cinerea* (Miler 2016) or *Myrmica rubra* (Leclerc & Detrain 2017). In *F. cinerea* moribund ants (CO^2 treated) were less likely to elicit rescue behaviour by nestmates when trapped by an antlion. The underlying mechanisms regulating this decision remained unexplained though. In *M. rubra* infected ants seem to lose the capability of processing social cues or nestmate recognition, thus becoming unsociable and leaving the nest (Leclerc & Detrain 2017). This could also explain our observations in heavily injured ants.

It appears that heavily injured ants first try to return to a resting position before eliciting a help pheromone or responding to nestmates. Thus offering a simple unconscious regulatory mechanism to distinguish between injury severity: if an ant can stand up its injuries are most likely not too severe, if it is unable to do so then it should not be rescued. The fact that all of these mechanisms/behaviours seem to be regulated through the injured ant and not by the helper exemplifies the importance of inclusive fitness in social insects to understand these observations.

Visual reinforcement of injury

We observed injured ants to move considerably slower near nestmates (the returning raid column). The visual capabilities and resolution of *M. analis* are still unknown, but from personal observations we think it unlikely for the ants to actually differentiate between a healthy and an injured individual solely by vision. A possible explanation for the slower movement could be the increased likelihood of being picked up by interacting with all passing nestmates (thus increasing the encounter possibility of a potential carrier). Furthermore, if the help pheromone is released, a stationary source should be easier to detect (by following the pheromone gradient) than a moving one. Interestingly injured ants are capable of reaching running speeds similar to that of the column when alone, suggesting that they should be able to keep up with the group (Fig. III.4). One should however note that observed speeds were collected under stress for what is most likely maximum running speeds, which the ants might not be able to keep up for the entire distance to the nest and which should be energetically costly. In addition when returning to the nest with a fresh wound we often observe the ants placing the cut off limb on the ground, thus increasing the risk of infection, this could be minimized by being carried back and staying immobile while waiting for help.

While comparisons to human behaviour and “acting more injured” near conspecifics are easy to make we want to emphasize that this is not the case here. This behaviour cannot be considered cheating (Riehl & Frederickson 2016), since all these ants are truly injured and not only benefit themselves from being carried back, but so does the colony (by reducing foraging costs/mortality) (Frank et al. 2017). The fact that heavily injured ants do not seem to call for help further underscores the argument against cheating (Fig. III.3*A*).

CONCLUSION

We describe in this study social wound treatment in insects through a multifaceted help system focused on injured individuals. This novel mechanism is not only limited to selective rescue of lightly injured individuals but moreover includes a differentiated treatment inside the nest that significantly reduces mortality. We further show that most decisions on who to treat or rescue are not made by the helper but unconsciously regulated by the injured ant. This study exemplifies the importance injured individuals play in a social species that hunts highly defensive prey. To minimize these costs adaptations occurred both on the social level (rescue and treatment) and the individual level (wound sealing/clotting).

SUPPLEMENTARY MATERIAL III

Fig. III.S1. Pictures of injury treatment. (A) A nestmate grabs a freshly cut off extremity of an injured individual. **(B)** The mouthparts of a nestmate enter a fresh wound repeatedly while holding the leg in place with the mandibles, antenna and front legs. **(C)** Ant that got bitten by a termite with the termite head remaining after the body was removed by its nestmates.

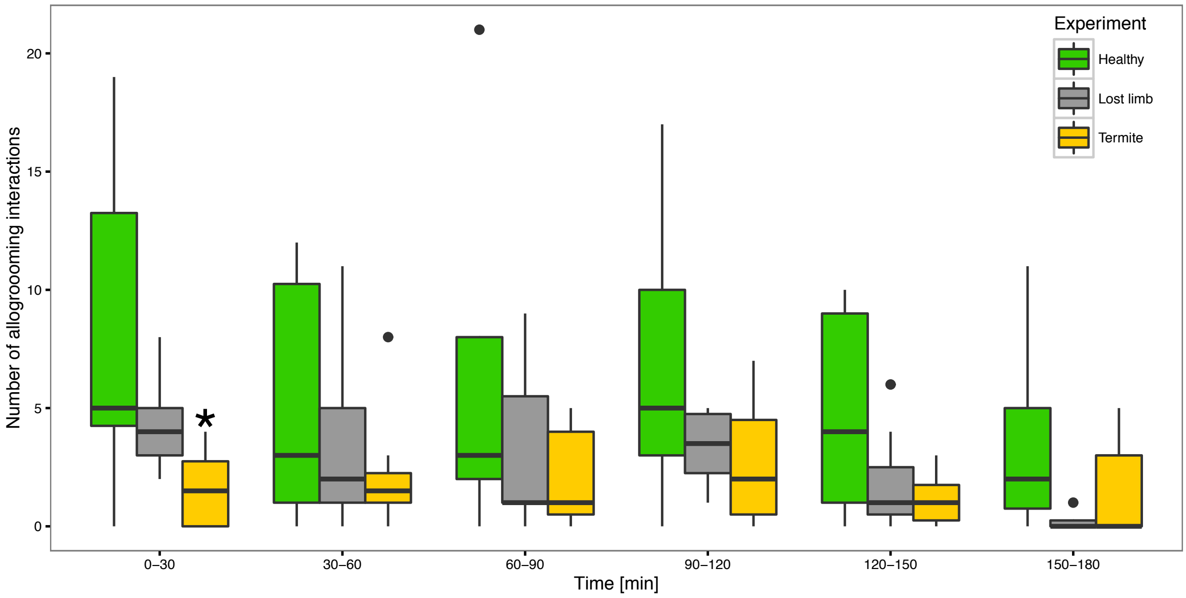

Fig. III.S2. Number of allogrooming interactions by nestmates. Number of allogrooming interactions by nestmates for differently treated individuals inside the nest in 30-minute blocks. Lost limb: injured ants with two cut off limbs (*N*=10); Termite: ant with dead termite soldier clinging on to it (*N*=10); Healthy: control experiment with healthy ant (*N*=6). Kruskal-Wallis rank sum test followed by a Dunn’s test with Holm-Bonferroni correction for the different time blocks. See also table III.S4 for detailed statistical results.

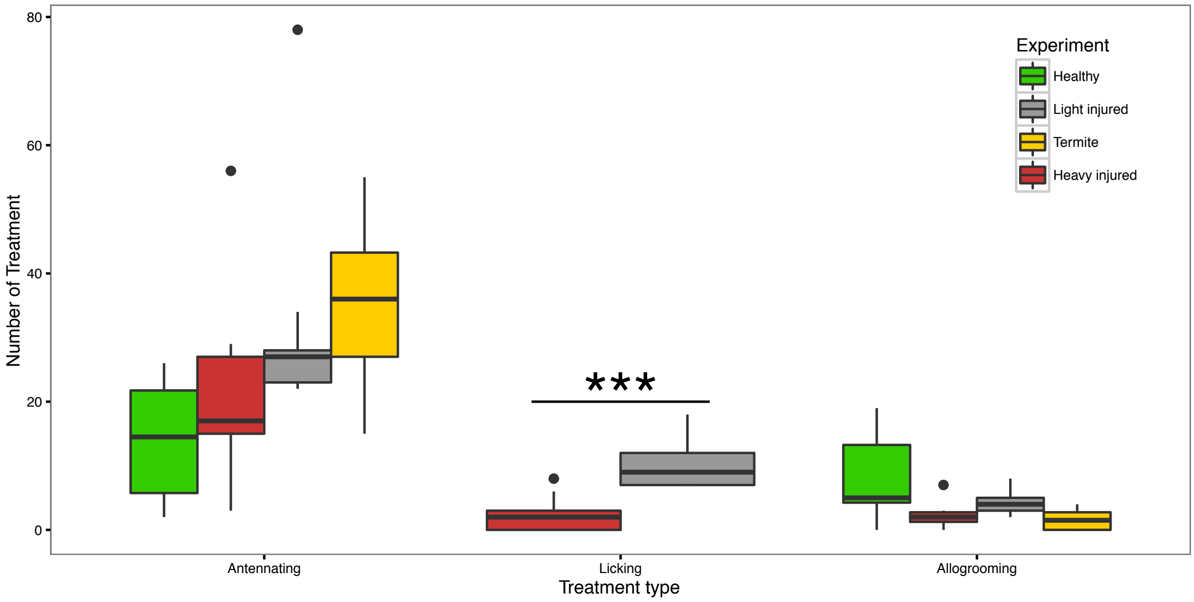

Fig III.S3. Ethogram of heavily injured ants together with the other manipulation trials. Number of behaviours shown by nestmates within the first 30 minute block for the different experiments. Light injured: injured ant with two cut off limbs (*N*=10); Heavy injured: injured ant with five cut off limbs (*N*=9); Termite: ant with dead termite soldier clinging on to it (*N*=10); Healthy: control experiment with healthy ant (*N*=6).

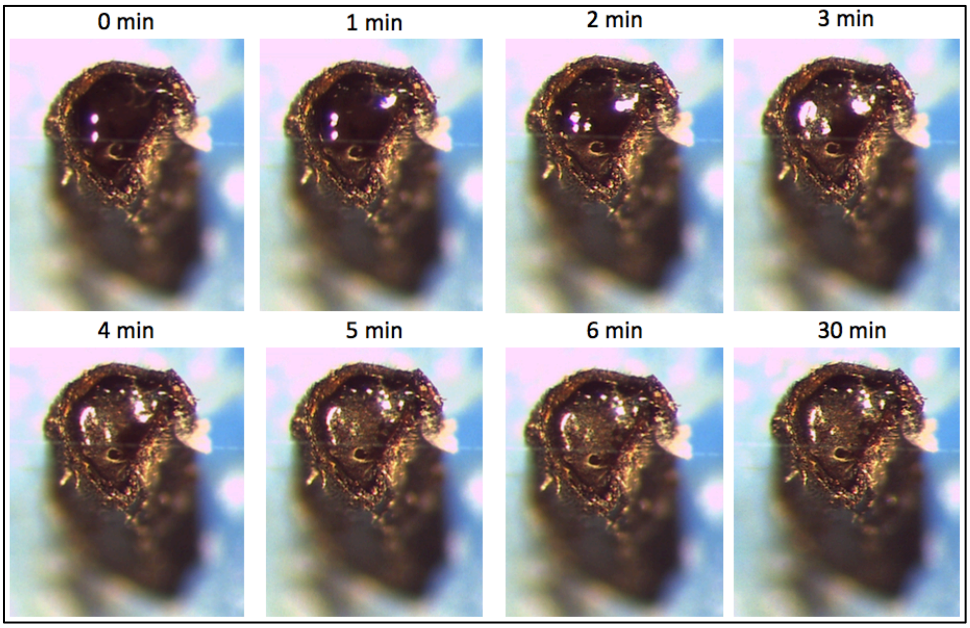

Fig. III.S4. Wound sealing in *M. analis*. Picture series of an open wound at the femur of a *M. analis* major.

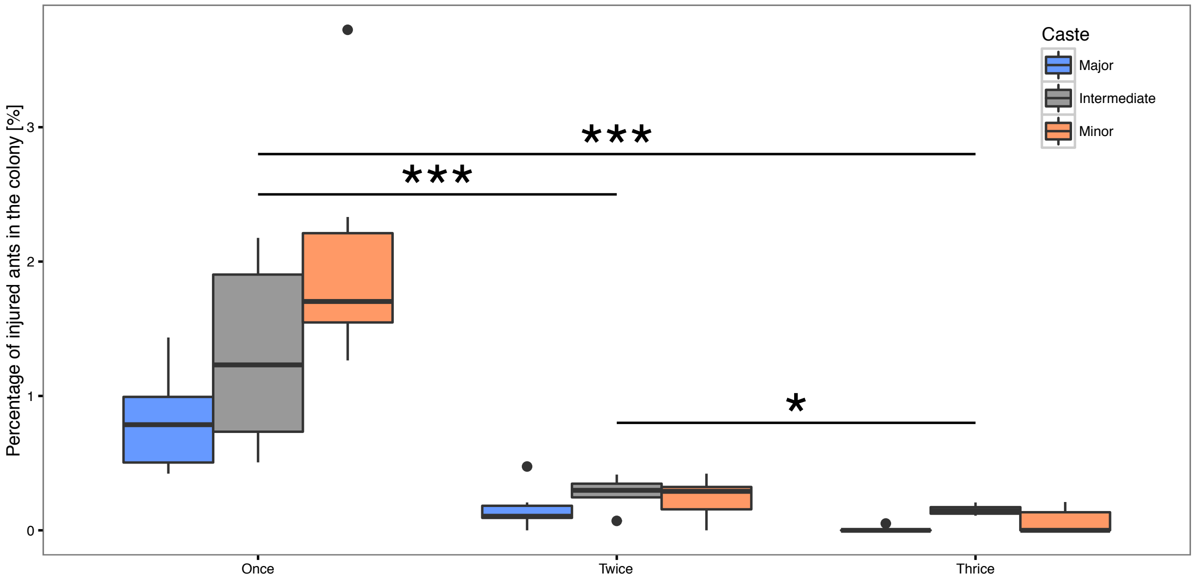

Fig. III.S5. Percentage of injury severity in the colony within each caste. Ants from six colonies were closely inspected for injuries and size class (major, intermediate, minor) and classified into three categories: once: one lost limb (*N*=292); twice: two lost limbs (*N*=46); thrice: three lost limbs (*N*=17), rest was healthy (*N*=6885). Significant values were measured for differences between the total percentage of injured ants within each injury severity class. *=p<0.05; ***=p<0.001

Table III.S1. Statistical differences in antennations by nestmates on differently injured individuals as shown in Fig III.1*A*. Kruskal-Wallis rank sum test followed by Dunn's test with Holm-Bonferroni correction). Abbreviations: Light = Ant with two legs cut off (*N*=10); Termite = Ant with termite clinging on body (*N*=10); Healthy = Control with an uninjured ant (*N*=6).

Time [min]	**State 1**	**State 2**	***Z***	***P***
0–30	**Kruskal-Wallis test** (X^2=9.07)			0.01**
	Healthy	Lightly	-2.12	0.03*
	Healthy	Termite	-2.99	0.004**
	Lightly	Termite	-0.93	0.18
30–60	**Kruskal-Wallis test** (X^2=6.25)			0.04*
	Healthy	Lightly	-1.68	0.09
	Healthy	Termite	-2.47	0.02*
	Lightly	Termite	-0.77	0.2
60–90	**Kruskal-Wallis test** (X^2=3.09)			0.21
90–120	**Kruskal-Wallis test** (X^2=1.50)			0.47
120–150	**Kruskal-Wallis test** (X^2=0.65)			0.72
150–180	**Kruskal-Wallis test** (X^2=0.98)			0.61

Table III.S2. Statistical differences in injury licking by nestmates on injured, as shown in Fig III.1*B*. Kruskal-Wallis test (X^2_5=22.71, $P<0.001$) followed by a Dunn's test with Holm-Bonferroni correction; *N*=10.

Time 1	Time 2	*Z*	*P*
0–30	30–60	2.48	0.08
0–30	60–90	2.80	0.03*
0–30	90–120	2.38	0.09
0–30	120–150	3.55	0.003**
0–30	150–180	4.20	<0.001***
30–60	60–90	0.30	1
30–60	90–120	0.01	0.50
30–60	120–150	1.01	0.94
30–60	150–180	2.03	0.21
60–90	90–120	-0.28	0.78
60–90	120–150	0.71	0.96
60–90	150–180	1.77	0.31
90–120	120–150	0.96	0.84
90–120	150–180	1.96	0.22
120–150	150–180	1.17	0.85

Table III.S3. Statistical differences in termite interactions by nestmates on handicapped individuals, as shown in Fig III.1*C*. Kruskal-Wallis test for termite biting (X^2_5=9.87, *P*=0.08). Kruskal-Wallis test for termite pulling (X^2_5=18.63, *P*<0.001) followed by a Dunn's test with Bonferroni-correction; n=10.

Time 1	Time 2	*Z*	*P*
0–30	30–60	1.82	0.34
0–30	60–90	2.96	0.02*
0–30	90–120	2.99	0.02*
0–30	120–150	3.58	0.003**
0–30	150–180	2.78	0.03*
30–60	60–90	1.15	0.88
30–60	90–120	1.18	1
30–60	120–150	1.82	0.38
30–60	150–180	1.15	0.99
60–90	90–120	0.03	0.49
60–90	120–150	0.70	1
60–90	150–180	0.11	1
90–120	120–150	0.67	1
90–120	150–180	0.08	0.94
120–150	150–180	-0.54	1

Table III.S4. Statistical differences in allogrooming interactions by nestmates on differently injured individuals as shown in Fig. III.S2. Kruskal-Wallis test (X^2_3=22.0, *P*<0.001) followed by a Dunn's test with Bonferroni-correction if significant). Abbreviations: Light = Ant with two legs cut off (*N*=10); Termite = Ant with termite clinging on body (*N*=10); Healthy = Control with an uninjured ant (*N*=6).

Time [min]	State 1	State 2	*Z*	*P*
0–30	**Kruskal-Wallis test** (X^2=9.43)			0.01**
	Healthy	Lightly	0.66	0.26
	Healthy	Termite	2.77	0.008**
	Lightly	Termite	2.36	0.018*
30–60	**Kruskal-Wallis test** (X^2=0.51)			0.77
60–90	**Kruskal-Wallis test** (X^2=1.22)			0.54
90–120	**Kruskal-Wallis test** (X^2=1.62)			0.44
120–150	**Kruskal-Wallis test** (X^2=1.97)			0.37
150–180	**Kruskal-Wallis test** (X^2=2.36)			0.31

Table III.S5. Statistical differences for mortality of differently treated ants as shown in Fig. III.2. Cox proportional hazards regression model: *P*<0.001.

Treatment	coef	*Z*	*P*	n
Injured	3.55	3.43	<0.001***	20
Sterile Control	0.74	0.6	0.55	20
Sterile Injured	1.68	1.54	0.12	20
Treatment	0.72	0.59	0.56	20

Table III.S6. Statistical differences in significance of rescue behavior compared to behavior on healthy individuals as shown in Fig. III.3*A*. Fisher's exact tests for count data between treatment healthy (no help) and the other categories with Bonferroni correction (*N*=20 per test). Abbreviations: Broken = Ant with 6 incapacitated legs; Pheromone = Heavy injured coated with synthesized help pheromone (DMDS/DMTS); Dummy= dead ant (frozen) coated with synthesized help pheromone (DMDS/DMTS); For explanation of treatments see Fig. III.5 *A-D* description.

Treatment	**P**	**n**
Lightly injured	0.001**	20
Heavyly injured	1	20
Broken	0.49	20
Pheromone	0.003**	20
Dummy	0.23	20

Table III.S7. Statistical differences in investigation time by nestmates on differently injured individuals as shown in Fig. III.3*B*. Kruskal-Wallis test (X^2_3=22.0, *P*<0.001) followed by Dunn's test with Bonferroni-correction; *N*=20 per test. Abbreviations: Fresh = Ant that freshly lost a leg on each side; Old = Same ant 24 hours later; Healthy = Control with an uninjured ant.

State 1	**State 2**	***Z***	***P***
Lightly	Heavily	-2.45	0.043*
Lightly	Dead	1.82	0.21
Lightly	Helped	1.35	0.53
Heavily	Dead	4.27	<0.001***
Heavily	Helped	3.80	<0.001***
Dead	Helped	-0.47	1

Table III.S8. Statistical differences in running speed by injured and healthy ants alone and near nestmates, as shown in Fig III.4. Kruskal-Wallis test (X^2_3=29.34, *P*<0.001) followed by Dunn's test with Bonferroni-correction; *N*=20 per test.

State 1	**State 2**	***Z***	***P***
Healthy alone	Injured alone	2.41	0.048*
Healthy alone	Healthy column	2.30	0.06
Healthy alone	Injured column	5.39	<0.001***
Injured alone	Healthy column	-0.11	1
Injured alone	Injured column	2.98	0.009**
Healthy column	Injured column	3.10	0.006**

CHAPTER IV: OPTIMAL FORAGING AND DECISION MAKING

As published in: Frank ET, Linsenmair KE (2017). Individual versus collective decision making: optimal foraging in the group-hunting termite specialist *Megaponera analis*. *Animal Behaviour* 130:27–35

ABSTRACT

Collective decision-making is one of the main mechanisms of organization in social insects. However, individual decision-making can also play an important role, depending on the type of foraging behaviour. In the termite-hunting ant species *Megaponera analis* information about foraging sites is collected by only a handful of individual scouts that have to convey this information to the colony as accurately as possible to optimize their foraging behaviour. We therefore looked at predictions made by optimal foraging theory to better understand the interplay between collective and individual decision making in this obligate group-raiding predator. We found a clear positive relation between raid size (200–500 ants) and termite abundance at the foraging site thereby confirming predictions of the maximization of energy theory. Furthermore, selectivity of the food source increased with distance, thus confirming central place prediction theory. The confirmation of these theories suggests that individual scouts must be the main driver behind raid size, choice and raiding behaviour. The marginal value theorem was also confirmed by our results: time spent at the hunting ground increased with distance and prey quantity. This raises questions on how foraging time at the food source is regulated in a group-hunting predator. Hunger decreased selectivity of scouts with respect to food sources, while average raid size increased and more scouts left the nest in search of prey, thus implying that scouts are aware of the hunger state of the colony and adapt their decision making accordingly. Remarkably, most central place foraging behaviours in *M. analis* were not achieved by collective decisions but rather by individual decisions of scout ants. Thus, 1% of the colony (10–20 scouts) decided the fate and foraging efficiency of the remaining 99%.

INTRODUCTION

By making collective decisions, group-living animals can increase the accuracy of a decision, at the cost of time (Chittka et al. 2009). Collective decisions have been extensively studied in social insects and are considered one of the main mechanisms for regulating behavioural choices, such as nest or food choice (Hölldobler & Wilson 2008, Mallon et al. 2001). For certain species, however, the choice of a food source cannot be taken by collective decisions. The foraging success of species that rely on independent foragers directly depends on the individual decisions they make (Pohl & Foitzik 2011). In species in which a large portion of the colony forages independently, the mistakes made by individuals have little impact. However, in species in which the colony depends on scouts for obtaining accurate information to send large numbers of individuals to the food source, mistakes become far riskier. We therefore looked at the foraging behaviour of the termite-hunting ant *Megaponera analis* to better understand the interplay between collective and individual decision making in their raid choice and the precision of these choices.

Megaponera analis is a strictly termite-eating ponerine ant species found in sub-Saharan Africa from 25°S to 12°N (Schmidt & Shattuck 2014), specialized in raiding termites of the subfamily Macrotermitinae at their foraging sites (Lepage 1981, Levieux 1966). Colony size varies between 440 and 1400 adult ants (Lepage 1981). 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. This searching phase can last up to 1 h and in unsuccessful cases the scout returns to the nest by a circuitous route (Longhurst & Howse 1979). Once a scout ant has found a potential site it investigates it, while avoiding contact with the termites. After this investigation, the scout returns by a direct route to the nest to recruit nestmates for a raid (Longhurst & Howse 1979). It recruits approximately 200–500 nestmates and leads them to the termites in a column-like march formation (Bayliss & Fielding 2002, Longhurst & Howse 1979). During the raid, division of labour occurs (Corbara & Dejean 2000): larger ants (majors) break open the protective soil cover created by the termites while the smaller ants (minors) rush into these openings to kill and pull out the prey (Villet 1990). After 13–20 min the hunt ends and the ants start collecting the dead termites and injured ants in need of assistance (Frank et al. 2017, Yusuf et al. 2013). Majors grasp up to seven termites between their mandibles and minors grasp up to three; the majority, however, return without termites (Yusuf et al. 2013). After collecting the termites, the column forms again and the hunting party returns to the nest together. *Megaponera analis* seems to show certain prey preferences, preferring to attack termite species that are easier to hunt and that have weaker defence capabilities (Yusuf et al. 2013).

The high variance in raid distances and raid sizes made us wonder how well the scouts used the information gathered at the prey site and how accurate these decisions were in the light of optimal foraging theory. This theory has been applied to various animals to understand their foraging patterns and behaviours (Macarthur & Pianka 1966, Olsson et al. 2008, Pearce-Duvet et al. 2011). Central place foraging theories expand upon present theories of optimal foraging to include animals that carry food back to a central place (Orians & Pearson 1979, Schoener 1979), as is the case for most ant species. We thus expected that increased accuracy of information conveyed by scouts would better match predictions of central place foraging theory. Additionally, although central place foraging theory has been extensively studied in honeydew-feeding ant species (Bonser et al. 1998) and solitary foraging ants (Pearce-Duvet et al. 2011) little is known about how well it predicts the foraging patterns of group-recruiting predatory ant species, such as *M. analis*. These ants behave markedly differently to other ant species in their raiding behaviour, although certain similarities to some bee species can be observed (Abou-Shaara 2014, Nieh 2004), which we address in the Discussion. Recruitment and foraging behaviour in ants are often regulated through pheromone trails. Food choices are passively regulated by the reinforcement of trails that lead to good food sources faster (Hölldobler & Wilson 1990). Predatory species need to minimize the time between the prey detecting them and capture of the prey (Witte et al. 2010). Widespread foraging strategies of predatory ants therefore include solitary foraging (most ponerines) and group foraging (as in driver ants) (Peeters & Crewe 1987); in both cases no time is lost trying to recruit nestmates. In group-recruiting predatory ant species only a very small percentage of the colony leaves the nest in search of food sources before recruiting a substantial portion of the colony to go out to exploit it. If their foraging behaviour is optimized in accordance with central place foraging theory, it would imply that the scouts exert extensive control on the colony, since only they hold all the information necessary for optimizing raiding behaviour. Decision making would therefore not be regulated collectively but by individuals. *Megaponera analis* is an ideal model organism to study these questions.

Predictions

We made the following predictions about the foraging behaviour of *M. analis* in the light of central place foraging theory.

(1) Animals should forage to maximize their net energy intake per unit feeding time, by minimizing the energy invested and selective food choice (Schoener 1971). We expected *M. analis* to select food sources with a high amount of energy gained per unit time (i.e. hunting as many termites as possible per ant in as little time as possible). Furthermore, to minimize energy investment, raid size should vary according to the size of the patch and the density of foraging termites (to optimize the ratio of termites per ant).

(2) As distance from the central place increases, selectivity should increase (Orians & Pearson 1979). Since food sources further away take more time and energy to reach, they should be richer in termites to compensate for the longer travel time. *Megaponera analis* should therefore only conduct raids to distant termite sources if they are of high quality.

(3) The optimal time a predator should spend at a food source before moving on to another site depends on the distance between feeding sites, if food intake rate at a hunting site decreases over time (Charnov 1976). Applied to the central place foraging theory this implies that the longer the travel time the longer one should exploit the food source before returning to the nest. *Megaponera analis* should therefore spend more time at feeding sites further away from the nest.

(4) Food selectivity depends on the consumer's hunger state, with increased food intake leading to increased diet selectivity and specialization (Macarthur & Pianka 1966, Schoener 1971). We expected *M. analis* to start conducting raids on less favourable termite sources if no successful raids occurred for a while and the hunger state of the colony was high. Furthermore, foraging behaviour was expected to become more risk prone (Cartar & Dill 1990).

A combination of laboratory and field experiments allowed us to test these predictions and shed light on the mechanisms regulating the foraging behaviour of group-recruiting predators. Do the individual scouts or collective decisions by the colony regulate it?

MATERIALS AND METHODS

Study area and organism

The study area is a humid savannah woodland 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 & Kampmann 2010). Colonies of *M. analis* were located in a radius of approximately 2000 m from the research station. The distances between the colonies varied between 10 and 200 m. Nests were most commonly located by following a raiding column or scout ant returning to the colony. In total 450 raids of 54 different colonies were observed. In these raids, the main termite species hunted was *Pseudocanthotermes* sp. but raids on *Macrotermes bellicosus* were also observed. Of these 450 field raids, data on the raiding parameters for this study were collected in 144 raids (134 for undisturbed colonies and 10 for hungry colonies). Colony size for 12 excavated colonies was between 900 and 2300 ants, a result comparable to previous studies in other regions (Villet 1990, Yusuf et al. 2013).

Data collection

Observations in April 2013 established that raiding activity was highest in the morning and afternoon hours between 0600 and 1100 hours and between 1500 and 1900 hours local time, which corresponds to previous observations (Bayliss & Fielding 2002, Longhurst & Howse 1979, Yusuf et al. 2014). Night raiding was also observed, but was not included in this study. Experiments and observations in the field were therefore carried out from 0700 to 1100 hours and from 1500 to 1800 hours from April to September 2013, August to October 2014, January to March and July to September 2015 as well as 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 majors (head width > than 2.40 mm), minors (head width < 1.99 mm) and intermediates (head width 2.40–1.99 mm) as proposed by Villet (1990).

Laboratory colonies

Four excavated colonies were placed in artificial nests in the field stations' laboratory. The nests (20x20 cm and 10 cm high), made of PVC plastic, were connected to a 1x1 m arena.

To enable raids, this arena was connected to a second arena (1x1 m) by a corridor either 10 or 30 m long. The ground was covered with soil from the natural habitat around the field station. The experiments were conducted after giving the ants 7 days to habituate to the artificial nests. Between experiments each ant colony was fed twice a day in the first arena, with a total of 300 termites per day, to ensure constant conditions. For the experiments *M. bellicosus* termites were placed in the second arena. These termites were found by scouts and triggered raiding behaviour on which we performed the experiments. Termites were placed in the second arena either at 8:00 or 16:00 and observed until a raid occurred or until 3 h elapsed. All laboratory experiments were carried out three times per colony (for a total *N*=12 per experiment). Owing to time constraints, the hunger experiments for large and small hunting grounds could only be done five times each with three colonies. All colonies were released into the wild at the end of the study (to their original nest location). For further details on laboratory keeping see Yusuf et al. (2013).

Although our results showed that field raids were predominantly conducted on *Pseudocanthotermes militaris*, we also observed raids on *M. bellicosus* in the field. We can thus safely assume that the species predated in the laboratory, *M. bellicosus*, is not an unnatural prey choice. We chose *M. bellicosus* as the prey species because of its ease of collection (collected from the surrounding area by attracting them with pots filled with dry grass overnight). Both species, *M. bellicosus* and *P. militaris*, forage principally on wood, grass or litter on the surface and cover their food with a thin layer of soil. The main difference between these species is the larger worker and soldier size of *M. bellicosus*. This might have affected the injury rate of *M. analis* during a raid, although this was not analysed in this study, but see Frank et al. (2017) for details of injured ants.

Experiments

Data on raid size (number of ants participating in a raid) and termite-carriers (number of ants carrying termites on the return journey) were collected by counting the individuals of the raiding column on the outward and return journeys. The durations of the different raid phases (outward journey, time at the hunting ground and return journey) were also timed. Hunting time was defined as the time between the arrival of the ants at the food source and the start of the departure of the return column.

In the field, distance to the termite-feeding site was measured by using a 40 m long measuring tape. In the laboratory, distance to the food source was either 10 or 30 m.

The termite abundance in the laboratory experiments was manipulated by offering either 50 termites in an area of 50 cm^2 (small hunting ground) or 250 termites in an area of 1260 cm^2 (large hunting ground). The areas where the termites were released were closed off with plastic barriers, thus preventing them from leaving the designated hunting area. Furthermore, the area where the termites were released was kept moist and had dry grass on it (in contrast to the surrounding area, which consisted of dry soil) so that the termites could build galleries. The termites were given 1–2 h to build their galleries before the ants gained access to the arena. Scout numbers were quantified by counting the ants outside the nest before each experiment. This number was only a conservative estimate as individual scouts entered and left the nest frequently, making it impossible to count them accurately (i.e. we could not tell whether a new scout left the nest or whether it was one we had previously seen entering the nest).

The hunger state of the colonies was manipulated by preventing raids for 48 h. In the field, this was done by placing 30 cm high PVC walls around the nest, which were dug 10 cm into the ground (to include all nest entrances within the enclosure). Observations throughout the starvation period confirmed that the scouts were unable to leave the enclosure (they were observed to search for exits during the activity period). In the laboratory, colonies were simply not fed for 48 h.

Statistical analysis

For statistical analysis and graphical illustration, 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). 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. If this was not the case a Kruskal–Wallis rank sum test was used, followed by a Dunn’s test with Holm–Bonferroni correction for post-hoc analysis. For comparisons between two non-parametric groups a Wilcoxon rank sum test was used. Linear correlations were calculated with a Pearson correlation test. Median values in the text are followed by a median absolute deviation.

RESULTS

Raiding behaviour

In the field, raids of *M. analis* occurred two to five times a day per colony. Raiding activity was highest in the morning and afternoon but also seemed to be strongly influenced by temperature, rainfall and humidity. During the dry season (January–March) raiding activity was more pronounced during the night. The main termite species hunted in our study site was *P. militaris*, making up 95% of the analysed raids (*N*=134 raids).

Maximization of energy and central place prediction

In the field, raids of *M. analis* varied in size between 50 and 800 ants conducting raids up to 48 m from the nest (Fig. IV.1). The median percentage of ants carrying termites back to the nest after a raid was 29 ± 19% (efficiency), of which each individual carried a median of 3 ± 2 termites (*N*=50 termite-carriers). The percentage of termite-carriers was not significantly influenced by either distance or raid size (ANOVA: $F_{2,116}$=0.18, *P*=0.84). Small raids (less than the median raid size of 318) conducted to distant locations (further than half the maximum raid distance, i.e. >24 m) made up only 2.2% of the raids (three of 134 raids; Fig. IV.1, lower right quadrant).

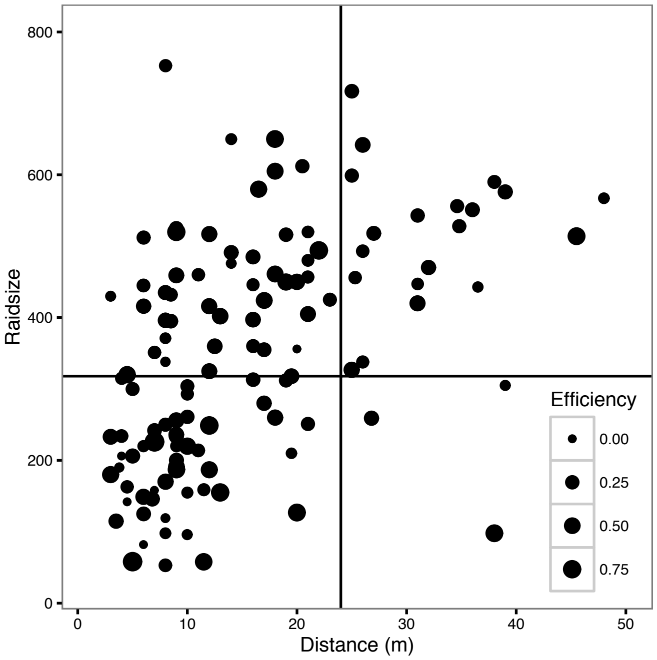

Figure IV.1. Correlation between raid size and distance. Efficiency is the proportion of ants carrying termites after the raid; dot size corresponds to the proportion of efficiency. Raid size is the number of ants participating in the raid. *N*=134 raids. Horizontal line: median raid size (318 ants). Vertical line: half of the maximum raid distance (24 m).

In the laboratory, raid size clearly correlated with termite abundance at the feeding site rather than distance (Fig. IV.2, Table IV.S1), with rich termite sites generally being exploited by large raids. Ant colony size also positively affected raid size (Fig. IV.S1*A*; Pearson correlation: r_{66}= 0.58, *P*<0.001). The percentage of the colony participating in a raid decreased with colony size. Small colonies conducted raids consisting of up to 75% of the colony, while larger colonies only allocated 35% of the colony to a raid (Fig. IV.S1*B*; Pearson correlation: r_{65}= -0.65 *P*<0.001).

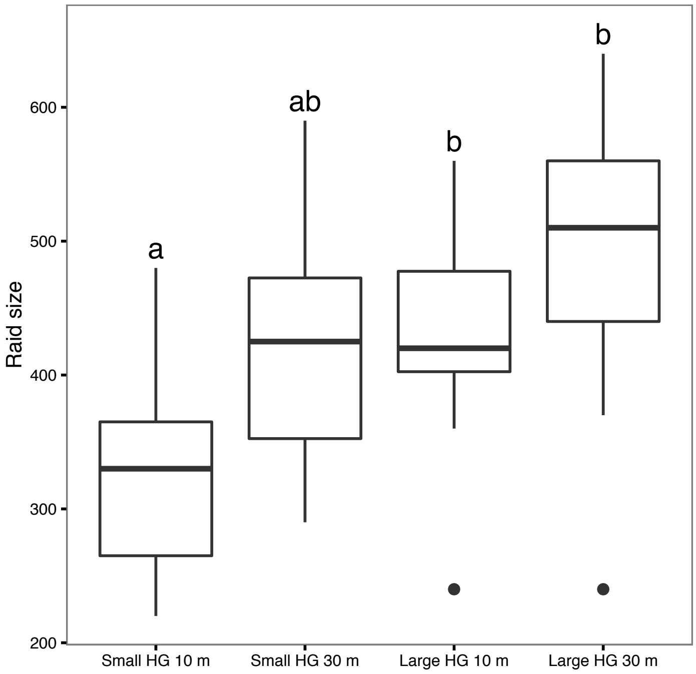

Figure IV.2. Raid size for differing distance and food quantity in the laboratory (*N*=12 per experiment). Small HG: small hunting ground (50 termites in an area of 50 cm^2); large HG: Large Hunting ground (250 termites in an area of 1260 cm^2). 10 m and 30 m: distance from the nest to the feeding site. Box plots 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; *P*<0.05).

Marginal value theorem

In the field, hunting time at the foraging site increased significantly with distance from the nest (Fig. IV.3*A*; Pearson correlation: r_{124}= 0.38, *P*<0.001). Hunting time was defined as the time between the arrival of the ants at the food source and the start of the departure of the return column. The number of termites killed per minute during the hunting period decreased with longer stays at the foraging site (Fig. IV.3*B*; Pearson correlation: r_{112}= -0.16, *P*=0.026). In the laboratory, hunting time also increased with food abundance and distance (Fig. IV.4, Table IV.S2).

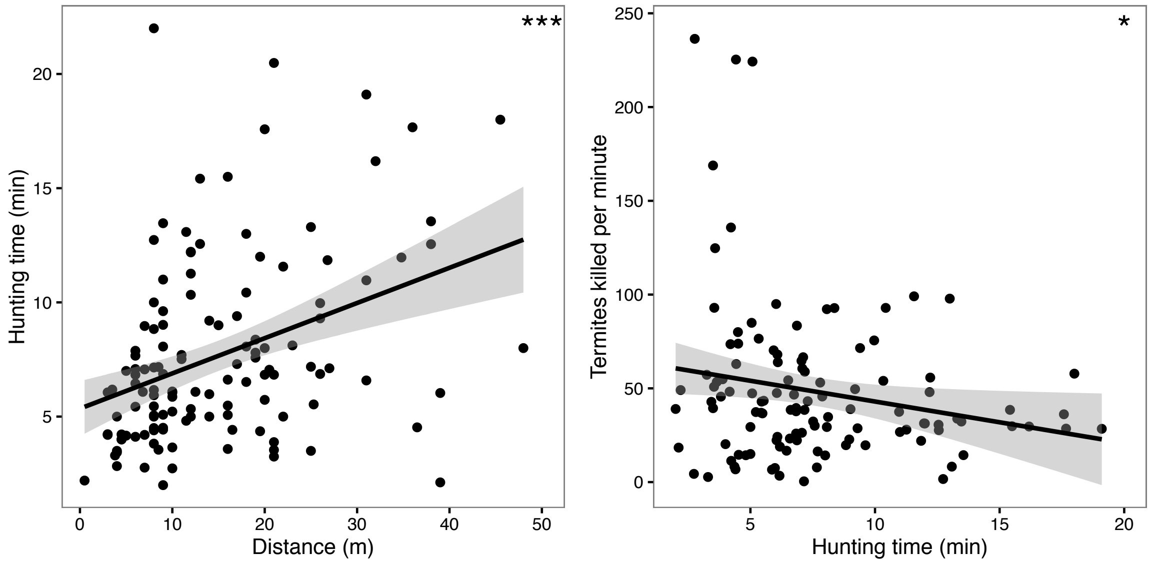

Figure IV.3. Relation of hunting time to distance and killing rate. A scatterplot is shown with linear regression line and 95% confidence interval (shaded area) for (a) time spent at the hunting ground in relation to distance from the nest and (b) the number of termites killed per minute during the hunting period in relation to the time spent at the hunting ground. *P<0.05; ***P<0.001.

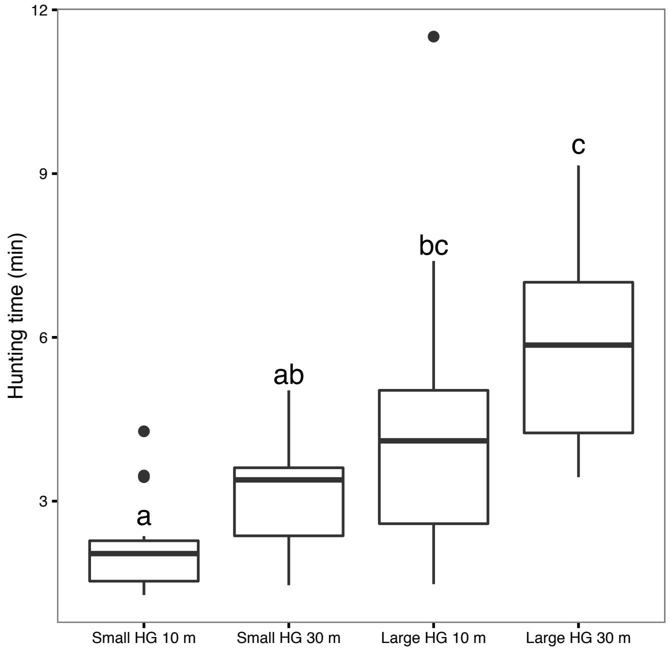

Figure IV.4. Hunting time for differing distance and food quantity in the laboratory (N=12 raids per experiment). Hunting time: time spent at the foraging site; small HG: small hunting ground (50 termites in an area of 50 cm^2); large HG: large hunting ground (250 termites in an area of 1260 cm^2). Box plots 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; P<0.05).

Hunger state prediction

In the field, starved colonies tended to conduct raids to significantly shorter distances (median 1.9 ± 0.9 m; *N*=10 raids) than control colonies (median 12 ± 8.9 m; *N*=134 raids; Wilcoxon test: *W*=1320, *P*<0.001). Raid size also varied strongly, with very small raids (50 ants) being conducted to very short distances (1 m; Fig. IV.S2). The number of scouts leaving the nest in search of food also clearly increased with increasing hunger, with two to three times as many scouts leaving the nest in starved colonies (30–50 scouts per colony versus 10–20 scouts in control colonies, although these numbers are only approximations, since the exact number could not be determined).

In the laboratory, our experiments showed that raid size for starved colonies was significantly larger than for normal raids (Fig. IV.5, Table IV.S3), both for small and large termite abundances at the hunting ground, although a larger sample size would be necessary to state this with certainty (Wilcoxon test: *W*=7, *P*=1, effect size=0.19; power=0.057). The time between the discovery of the food site by the scout and the start of the raid was also significantly shorter in starved colonies (425 ± 145 s) than satiated colonies (1040 ± 889 s; Wilcoxon test: *W* = 0.81, *P*<0.001).

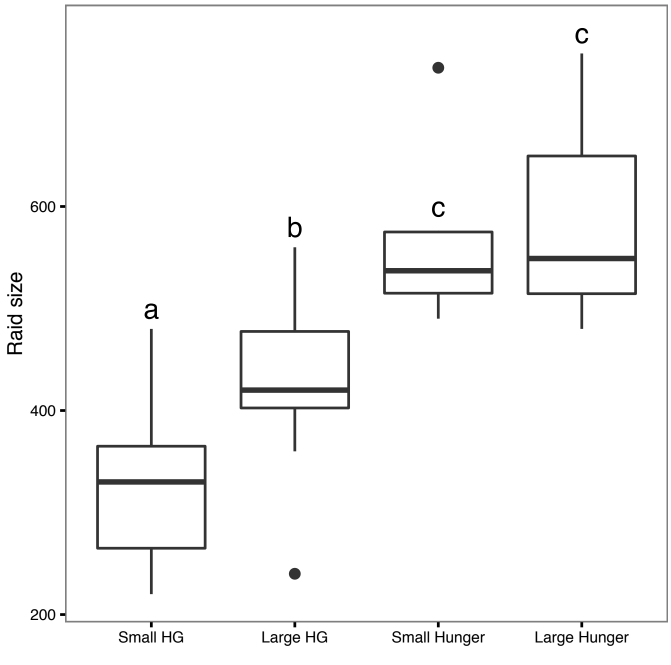

Figure IV.5. Raid size for hungry and satiated colonies in the laboratory. Small HG: small hunting ground (*N*=12 raids; 50 termites in an area of 50 cm^2); large HG: large hunting ground (*N*=12 raids; 250 termites in an area of 1260 cm^2); small hungry: colony starved for 48 h for small HG (*N*=5 raids); large hungry: colony starved for 48 h for large HG (*N*=5 raids). Box plots 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; *P*<0.05).

DISCUSSION

We confirmed various central place foraging theory predictions for *M. analis*. The scouts not only collected qualitative information about the food source, but also conveyed this information accurately to the colony. This gave us new insights into the importance of different factors for their raiding behaviour, such as termite quantity and distance. Remarkably, most of the decisions that optimized their raiding behaviour were regulated not collectively, but by individual scout ants.

Maximization of energy

In the laboratory, there was a clear positive relation between raid size and termite abundance at the foraging site thereby confirming predictions of the maximization of energy theory. These results support our hypothesis that scouts have to be able to assess the richness of a food source while examining it.

In the field, the median percentage of termite-carriers in a raid was 29% regardless of distance or raid size. Termites were very rarely observed to be left behind at the hunting ground (in any obvious quantity); we assume that the ants try to adjust their raid size to the richness of the food source, leading to only 30% of the ants having to transport back termites. The other ants have other essential roles in the hunting process, such as killing the termites and potentially also protecting the termite-carriers on the return journey. This was supported by experiments in the laboratory, with smaller raids only going for smaller hunting grounds (Fig. IV.2). We believe that the laboratory results can be extrapolated to the field since the information about patch quality, for regulation of raid size, was collected by scouts in the field and in the laboratory under similar conditions (the termites in the laboratory had 1–2 h to create foraging galleries prior to investigation by the scouts). Of course, once the actual hunt started these raids were markedly different; thus information on hunting time and percentage of termite-carriers should be compared with care.

Our field observations are in line with previous observations (Lepage 1981, Levieux 1966, Longhurst & Howse 1979). Other studies in Tanzania and Kenya seem to imply that colony size is the main factor in raid size variation (Bayliss & Fielding 2002, Yusuf et al. 2014). While this factor also played a role in our observations (Fig. IV.S1*A*), the variation in raid size within the colonies can probably best be explained by adaptation of the raid size to the termite foraging site. Intercolonial differences in raid size were also not proportionate to colony size. Large colonies seemed to conduct raids barely larger than those of small colonies (Fig. IV.S1*B*), suggesting that raid size is limited by other factors. Some possible limitations could be the recruitment capacity of the scout, raid organization or lack of richer/larger hunting grounds. Larger colonies seem to also have the advantage of conducting two raids simultaneously (personal observation).

Individual learning by the scouts might have influenced our results in the laboratory with the scouts learning the position of the food sources. Although this should not have influenced our main results, in the field the same site can also be exploited multiple times. The number of termites at the feeding site changes constantly, depending on humidity, time of day and activity of the termites; it is therefore still always necessary for the scout to properly assess the feeding site to adapt the raid size accordingly, which is also what we observed in the laboratory.

In ants, individuals can assess the quality/quantity of a food source to decide whether to recruit other ants or exploit the food source individually, thereby optimizing energy investment according to the food source (Hölldobler & Wilson 1990, Witte et al. 2010). In *M. analis* individual exploitation of a food source is not an option. Conducting large raids to small hunting grounds would lead to a waste of energy, unnecessarily occupying a portion of the colony. The ability of *M. analis* to adjust their raid size to the hunting ground allows a more flexible allocation of limited resources (ants) in the foraging process and relies on individual decisions made by scouts, which collect the necessary information.

Central place prediction

At short distances the full spectrum of raid sizes was observed. With increasing distance from the nest, the minimal raid size started to increase, thereby reducing the variance until 98% of the raids were large at the furthest distances (Fig. IV.1). In the previous section, we showed that raid size correlated positively with termite abundance at the foraging site. We therefore conclude that food selectivity increases with distance. Furthermore, our laboratory experiments showed that raid size depended on termite abundance at the foraging site and not on its distance (Fig. IV.2), thereby confirming central place foraging theory.

The efficiency of a single ant seemed to be independent of raid size or distance (Fig. IV.1). At the same distance a large raid to a large food source is as efficient as a small raid to a small food source, from an energetic point of view. Why then do the ants not conduct small raids to far distances? We may explain this pattern by looking at the foraging process from the perspective of the individual scout rather than the raid. Scouts are exposed to considerably larger risks when searching for food sources alone. Those that move further from the nest spend more time travelling and are therefore exposed to predation risks for longer. This increased risk can potentially only be outweighed if it leads to conducting raids to rich foraging sites.

Distance also seemed to affect raid size, although the significant differences were clearer when looking at the termite abundance at the food source. An outlier is the result for large raids conducted at far distances for poor termite sites in the laboratory (Fig. IV.2). It is worth keeping in mind, however, that the experimental set-up removed the possibility of choice, forcing the ants to exploit the presented food source. If no other choices are present it makes sense for the ants to exploit an, under natural circumstances, unfavourable food source.

In conclusion, these experiments allowed us to identify food source quality rather than distance as the main factor affecting raid size. However, we still do not fully understand the raiding behaviour in the light of present theories due to the lack of observations of small raids to far locations.

Marginal Value theorem

The hunting time at a food source in *M. analis* seemed to be in line with what the marginal value theorem predicts. Hunting time increased with distance (Fig. IV.3*A*) but killing/collecting speed decreased over time (Fig. IV.3*B*), while richer food sources were exploited longer than poorer food sources (Fig. IV.4).

The diminishing returns over time at a raiding site are likely to be more pronounced in termites than more passive food sources. Termites start to actively retreat into their galleries as soon as the ants arrive, with the soldiers staying behind to protect the retreating workers (Corbara & Dejean 2000). Nevertheless, a clear positive relation was observed between hunting time, distance and termite abundance, both in the laboratory and in the field. Since in the laboratory termites were unable to retreat, this may lead to less natural behaviour by the termites when attacked. Something to consider is also the handling time after the fight (i.e. collecting the dead termites); this phase is included in our definition of hunting time and could depend on the number of termites killed at the food patch. We believe that the handling time remained relatively constant since the raid size depended on the termite numbers (i.e. more ants were available to handle more termites). Furthermore, results for hunting grounds with the same termite numbers showed an increase in hunting time at greater distances (Fig. IV.4). We therefore believe that it is safe to assume that the hunting behaviour is in line with what the marginal value theorem predicts.

The marginal value theorem has been extensively studied in solitary foraging animals and ants (Olsson et al. 2008; Pearce-Duvet et al. 2011). Studies on ants mostly focus on single-prey loaders (Pearce-Duvet et al. 2011), in which the marginal value theorem does not apply (Charnov 1976). There are some studies on honeydew-feeding ants (such as *Lasius niger*, *Lasius fuliginosus*, *Myrmica ruginodis*), which have observed foraging strategies in line with the marginal value theorem (Bonser et al. 1998). Our study is the first, though, to show these patterns in a group-hunting predatory ant species such as *M. analis*. The processes that regulate this for group-foraging ants remain unclear. *Megaponera analis* has various reasons, such as predation risk, to return to the nest together and they take great care to do so (Bayliss & Fielding 2002; Longhurst & Howse 1979). We propose a quorum decision-making process as the regulating mechanism for the hunting time. The ants could have a certain termite encounter threshold, which at a certain point leads the ants to switch from hunting termites to collecting dead termites. Further experiments are necessary, though, to determine a clear regulatory mechanism for the hunting time.

Hunger state prediction

We observed that with increased hunger prey selectivity decreased and average raid size increased. We also observed more scouts searching for prey, in some cases doubling the number of scouts compared with satiated colonies.

Field experiments showed that raids were conducted to much shorter distances (1.9 ± 0.9 m versus 12 ± 8.9 m), some of them even being conducted to locations so near (ca. 1 m) that a trail to the termites was formed rather than a normal raid, with very few ants participating. Additionally, our laboratory experiments showed that hungry colonies always tended to conduct overly large raids (Fig. IV.5), with shorter investigation of the hunting ground by the scouts beforehand (425 ± 145 s versus 1040 ± 889 s for satiated colonies).

We hypothesize that a reduction in distance suggests that scouts become less selective in their prey choice, conducting a raid to the first termite location they find. This would imply that under normal circumstances scouts encounter termite foraging sites much more frequently but decide against exploiting them if they are too small. The reduced investigation time in hungry colonies probably make it harder for the ants to adapt their raid size to the feeding site (due to a lack of accurate information) and may explain the lack of raid size variation in hungry colonies. An alternative hypothesis could be that hungry ants in the colony are easier to recruit, thus always leading to larger raids for the same recruitment effort (Fig. IV.5).

These results suggest that foraging behaviour becomes more risk-prone under hunger, supporting hunger state prediction theory and observations in bumblebees, *Bombus terrestris* (Cartar & Dill 1990). Furthermore, bumblebees also became more sensitive to recruitment pheromones in hungry colonies (Molet et al. 2008). We hypothesize that by conducting large raids to feeding sites that, in principle, would not need it the colony invests a larger portion of its resources (ants) for the small chance of gaining more termites. The reduced food site selectivity and investigation time by the scouts suggest that they prefer to conduct a raid to a potentially unfavourable feeding site, thereby reducing the risk of being discovered at the cost of information. This implies an interesting interplay between collective and individual selectivity of prey choice. The colony would need to convey their hunger state to the scouts so that they can adapt their individual prey selectivity accordingly.

Individual decision-making

Most of our results suggest a high degree of control by individual scouts on the foraging behaviour of the entire colony. Individual scout ants regulated most optimal foraging behaviours observed in this study. Scouts have to be acutely aware of the hunger state of the colony to know how selective they can be with their prey choice. They need to collect sufficient information about a termite foraging site to decide whether to conduct a large or small raid, while keeping distance from the nest in mind. This information must then be passed on to the colony so that the appropriate raid can be conducted. Unlike honeybees, *Apis mellifera*, it is not necessary for the scouts to pass on detailed information about the location of the feeding site to the others, since the scout leads the column of ants to the termites. Thus, the only information that is vital during recruitment is the termite abundance at the food source, thereby varying the number of raiding ants. Scouts do not always succeed with recruitment after finding a foraging site (personal observation), suggesting that some control by the colony in the collective decision to conduct a raid is present. The number of scouts searching for food also seems to depend on the hunger state of the colony and is therefore probably regulated collectively, potentially through thresholds regarding hunger. Although it is possible that the hunting time at the foraging site is also regulated by scouts (by giving a signal when to stop), we believe that a collective decision by the raiding ants (quorum decision making) is more likely.

Scouts in social insects

The foraging behaviour of *M. analis* can to some extent be compared with that of slave-making ants (Pohl & Foitzik 2011). In the slave-making ant *Protomognathus americanus* the colony also depends on the accuracy of the decisions made by individual scouts for their raiding efficiency. Individual scouts can assess the size of a potential host colony and make their decision accordingly, with larger colonies having more pupae to be stolen (Pohl & Foitzik 2011). One big difference to *M. analis* is that these colonies only include a handful of ants (three to six slave-maker ants) versus over 1000 in *M. analis* colonies. Furthermore, just because prey can be hunted in a group does not imply that it will be. *Ophthalmopone berthoudi*, for example, is a solitary hunting termite specialist in the sister genus of *Megaponera* (Peeters & Crewe 1987). There are various strictly termitophagous ant species that hunt solitarily or in groups. It is unclear, though, how exactly these different strategies for the same food source evolved and there are likely to be various factors influencing it (Peeters & Crewe 1987).

Honeybees and some stingless bee species (Nieh 2004) also have a certain proportion of workers scouting for food while the rest wait in the nest to be recruited (Abou-Shaara 2014). Both *A. mellifera* and *M. analis* hunt a stationary but temporally variable food source: termite foraging sites do not normally change position if left undisturbed but are only active at certain times of day, similar to nectar and pollen in flowers. This is an important prerequisite since the time taken by a scout to return to the nest and recruit nestmates would be problematic when hunting mobile prey, although there are exceptions where scouts are present as in the ponerine ant *Leptogenys diminuta* (Witte et al. 2010). The necessity for scouts in group-hunting species can also be bypassed by directly foraging in groups, as in *Leptogenys* sp. in Cambodia (Peeters & deGreef 2015). These ants hunt large millipedes in groups of several dozen workers; subduing their prey would otherwise be difficult. Thus, the millipede can be overwhelmed immediately without first recruiting nestmates, which would increase the risk of losing such mobile prey (Witte et al. 2010).

One very clear difference between honeybees and *M. analis* is the ratio of scouts to recruits. In honeybees 5–25% of the workers act as scouts, foraging individually and recruiting if need be (Seeley 1983), while in *M. analis* scouts comprise roughly 1%. This stark difference could be due to the number of foraging trips. While *M. analis* will only exploit three to five food sources over a day, honeybees exploit dozens of different sources in the same time window (Abou-Shaara 2014) and more importantly are able to do so alone. Since *M. analis* requires only a few hunting trips per day, a much smaller number of scouts could suffice to find enough adequate food sources. This could be comparable to certain kleptoparasitic stingless bee species, such as the genus *Lestrimelitta*, which invade other bee nests for food (Gruter et al. 2016). Bumblebees offer an interesting example of a eusocial species foraging on stationary food sources without clear recruitment (Dornhaus & Chittka 2004a). The current scientific reasoning for this is that the benefits of recruitment decrease with increasing patchiness of food sources (Dornhaus & Chittka 2004b). Bumblebees forage mostly in open meadows in a very heterogeneous environment with many food sources (Dornhaus & Chittka 2004a), so while activation of nestmates can be observed, there is no need for direct recruitment to a specific location. Ultimately the foraging behaviour of *M. analis* can be compared with that of many other social insects (stingless bees, slave-making ants); further studies are necessary, though, to determine the factors that truly regulate scout numbers and group/solitary foraging in a cross-family comparison.

CONCLUSION

In conclusion, we were able to show that most central place foraging predictions are fulfilled by the raiding behaviour of *M. analis*. Remarkably, this is not only achieved by collective decisions but also by an interplay between individual decisions by scout ants and collective responses by the colony, thereby having 1% of the colony (10–20 scouts) potentially deciding the fate and foraging efficiency of the other 99%.

SUPPLEMENTARY MATERIAL IV

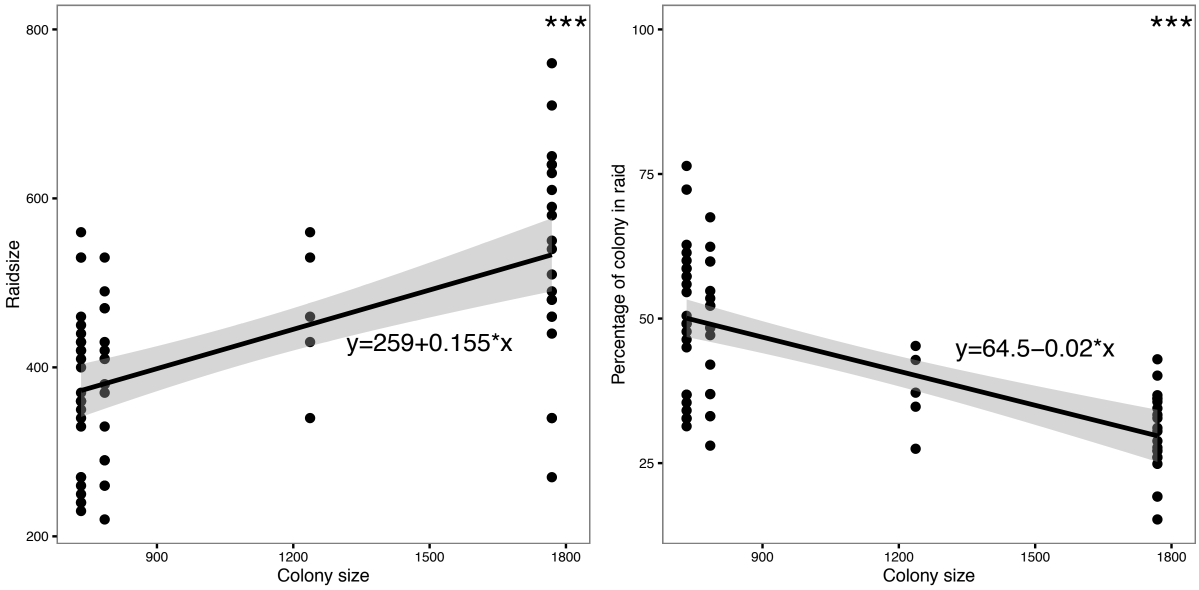

Figure IV.S1. Effects of colony size to raid size. (A) Raid size in relation to colony size and **(B)** percentage of colony participating in raid in relation to total colony size in the laboratory. Black line: linear regression line; shaded area: 95% confidence interval. ***$P<0.001$; $N=69$ raids.

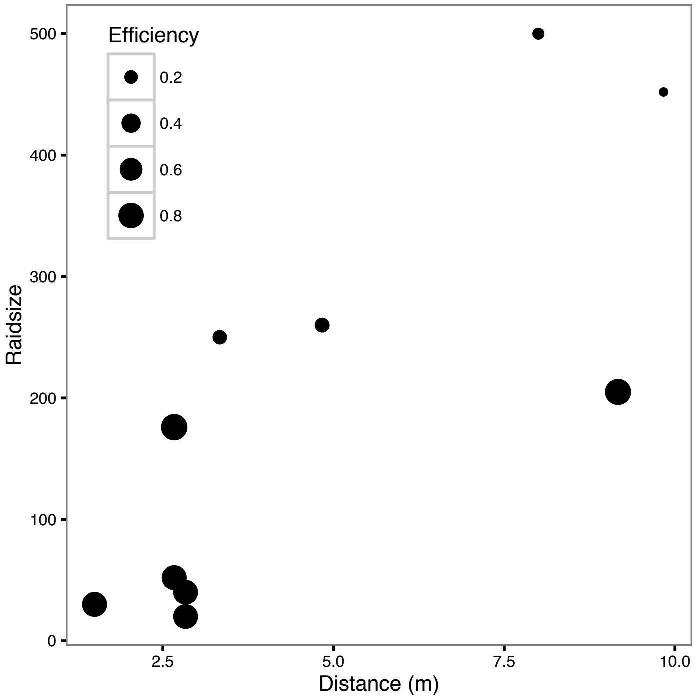

Figure IV.S2. Correlation between raid size and distance for starved colonies. Efficiency is the proportion of ants carrying termites after the hunt. Raid size is the number of ants participating in the raid. $N=10$ raids.

Table IV.S1. Statistical differences in raid size depending on distance and food quality as shown in Fig. IV.2. Kruskal–Wallis rank sum test (X^2_3=15.7, *P* <0.001) followed by Dunn's test. Small hunting ground (HG) 10 m: *N*=15 raids; Small HG 30 m: *N*=8 raids; Large HG 10 m: *N*=12 raids; Large HG 30 m: *N*=13 raids.

Caste 1	Caste 2	*Z*	*P*
Small HG 10 m	Small HG 30 m	-1.90	0.11
Small HG 10 m	Large HG 10 m	2.33	0.049
Small HG 10 m	Large HG 30 m	3.90	<0.001
Small HG 30 m	Large HG 10 m	0.15	0.44
Small HG 30 m	Large HG 30 m	1.44	0.15
Large HG 10 m	Large HG 30 m	-1.44	0.22

Table IV.S2. Statistical differences in hunting time depending on distance and food quality as shown in Fig. IV.4. Kruskal–Wallis rank sum test (X^2_3=24.7, *P* <0.001) followed by Dunn's test. Small hunting ground (HG) 10 m: *N*=15 raids; Large HG 10 m: *N*=12 raids; Small HG 30 m: *N*=8 raids; Large HG 30 m: *N*=13 raids.

Caste 1	Caste 2	*Z*	*P*
Small HG 10 m	Small HG 30 m	1.43	0.077
Small HG 10 m	Large HG 10 m	-3.06	0.001
Small HG 10 m	Large HG 30 m	4.80	<0.001
Small HG 30 m	Large HG 10 m	-1.23	0.11
Small HG 30 m	Large HG 30 m	-2.66	0.004
Large HG 10 m	Large HG 30 m	1.58	0.057

Table IV.S3. Statistical differences in raid size depending on hunger state and hunting ground as shown in Fig. IV.5. Kruskal–Wallis rank sum test (X^2_3=19.0, *P* <0.001) followed by Dunn's test. Small hunting ground (HG) 10 m: *N*=15 raids; Large HG 10 m: *N*=12 raids; Small hungry: *N*=5 raids; Large hungry: *N*=5 raids.

Caste 1	Caste 2	*Z*	*P*
Small HG 10 m	Large HG 10 m	-2.29	0.011
Small HG 10 m	Small hungry	-2.99	0.001
Small HG 10 m	Large hungry	-3.66	<0.001
Large HG 10 m	Small hungry	-1.56	0.05
Large HG 10 m	Large hungry	-1.88	0.03
Small hungry	Large hungry	-0.004	0.5

Returning raid column of *Megaponera analis*, notice the raid-guard standing on the right side of the picture.

CHAPTER V: TASK ALLOCATION AND RAID ORGANIZATION

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ABSTRACT

Division of labour 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 labour 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 & Wilson 1990). Worker division of labour can arise from combinations of worker age, morphology, frequency distribution and dominance interactions (Hölldobler & Wilson 2008). Within behavioural castes, like brood care or foraging, further task partitioning can occur (like brood care focused on grooming or feeding). This behavioural caste membership can have a physiological or developmental basis (Hölldobler & Wilson 2008). There is a variety of evidence and debate about how fluid movement between behavioural 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 & Wilson 2008). In addition to age polyethism, worker division of labour may also have a morphological basis (Hölldobler & 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 labour 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 analysed the raiding behaviour 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 & Howse 1979, Bayliss & 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 & 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 labour 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 & Linsenmair 2017a, Bayliss & Fielding 2002). These scouts always belong to the largest ants in the colony (Longhurst & 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 & Fielding 2002, Longhurst & 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 & Linsenmair 2017a). During the raid division of labour occurs (Corbara & 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 & 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 behaviour, 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 behaviour 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 & 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 analysed 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 & Howse 1979, Villet 1990), with newly defined behaviours, 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 & 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 & Fielding 2002, Longhurst & 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-colour code on the thorax (four colours used: red, blue, green, gold) depending on their position (front, centre, 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 behaviours 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, centre, 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; centre 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. V.1*A* and Table V.S1). 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. V.1*B* and Table V.S2).

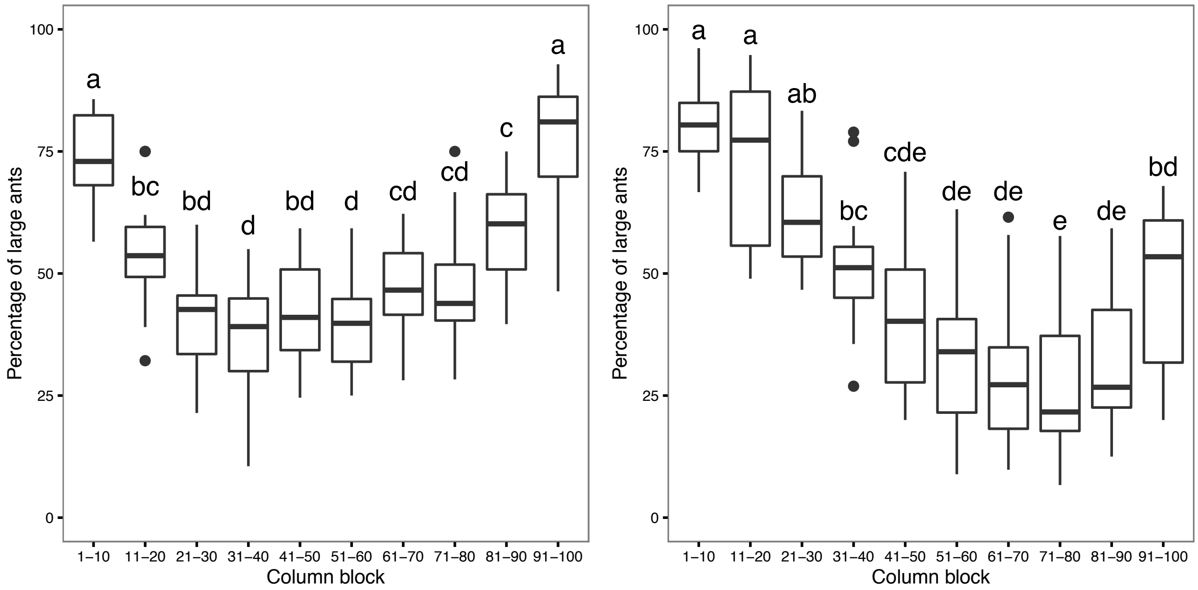

Fig. V.1. Raid column formation. Representation of the larger and smaller workers of *M. analis* within the raiding column in 10% blocks of the column, with 1-10% being the front and 91-100% being the last 10% of the column (see Table V.S1 and V.S2 for detailed statistical results). **(A)** Raid column on the way out to the termites. **(B)** Raid column on the return journey from the termites.

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 V.1).

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

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

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 behaviour 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 V.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 centre contributed 4 ± 5% and tail ants were never observed to lead a return column (*N*=5 raids; definition: front: first 10%; centre: 10-90%, tail: last 10% of the column).

Table V.2. Position of ants in the raid column. With front being the first 10%, tail being the last 10% and centre 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.

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

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. V.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. V.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. V.2).

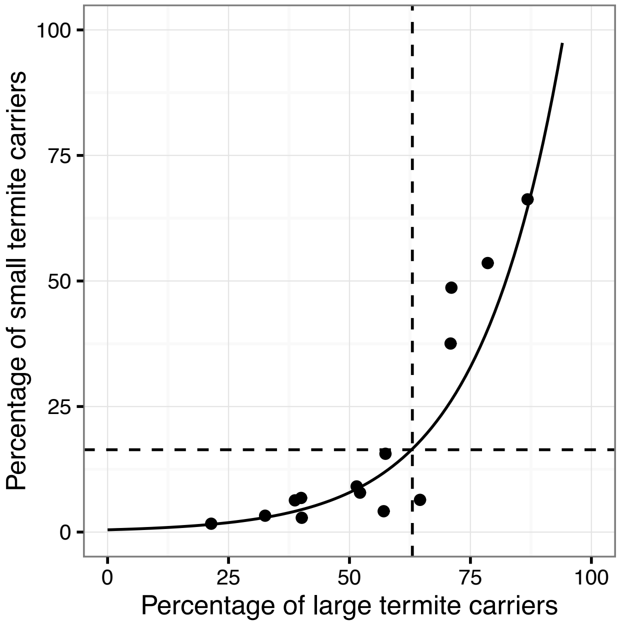

Fig. V.2. Relation of large to small termite carriers. Plot of small termite carriers against large termite carriers quantified during the return journey for differently saturated raids. Black line: exponential fit (formula: $y=a*e^{b*x}$). Dashed line intersection point: point were more small ants start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

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. V.3; Table V.S3 and V.S5). This formation could only clearly be recognized in raids that were not oversaturated with prey (<70% termite carriers; Fig. V.3*A*; Table V.S3 and V.S4). In oversaturated raids (>70% termite carriers) no clear formation was recognizable for the termite carriers (Fig. V.3*B*; Table V.S5 and V.S6).

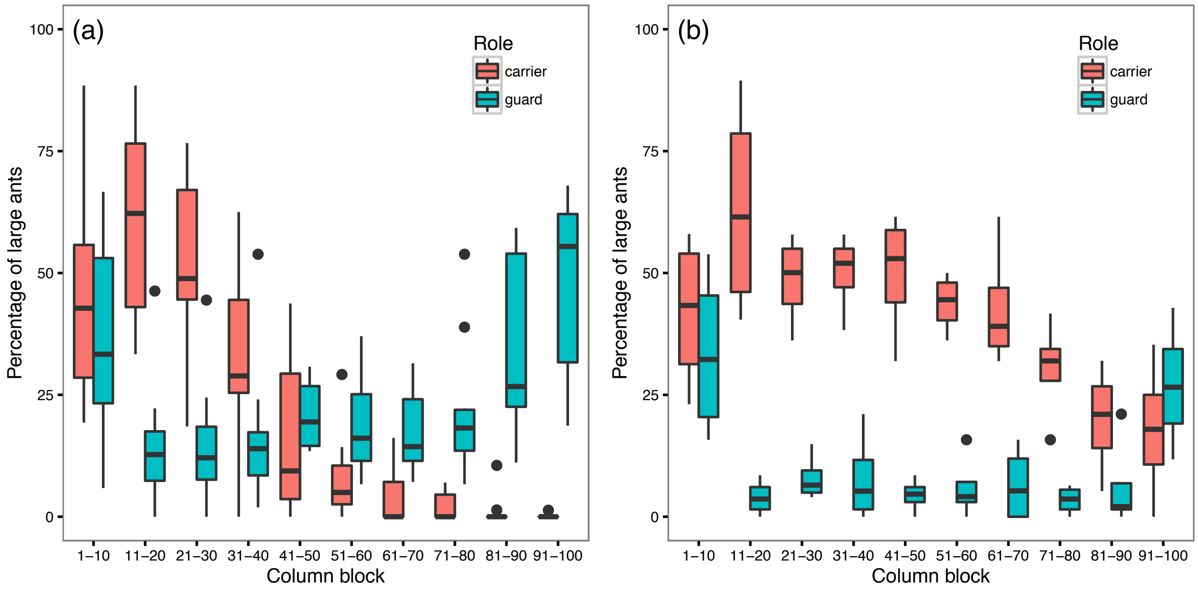

Fig. V.3. Formation of termite carriers and raid-guards in the returning raid column. Representation of the larger and smaller workers of *M. analis* within the raiding column in 10% blocks of the column, with 1-10% being the front and 91-100% being the last 10% of the column (see Table V.S3–S6 for detailed statistical results). **(A)** Normally saturated raid (<70% termite carriers). **(B)** Oversaturated raid (>70% termite carriers).

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 V.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. V.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 V.2).

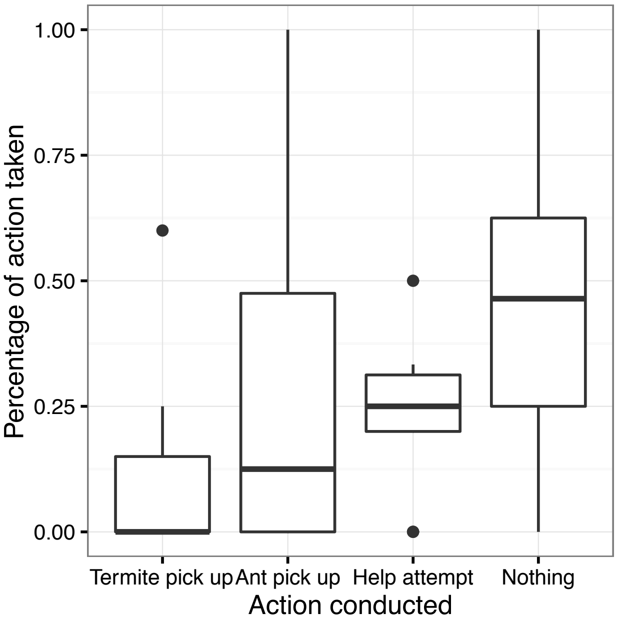

Fig. V.4. Percentage of actions observed in lingerer ants. Termite pick up: picking up a dead termite. Ant pick up: picking up an ant still at the hunting ground. Help attempt: trying to pick up an uncooperative ant. Nothing: not encountering any of the previous categories and leaving empty.

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. V.3). In addition to defence, 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 V.3 and Table V.S7). On the return journey these ants were at the front (Table V.3 and Table V.S7) but over time started to fall back, showing no clear position fidelity by the time the column was quantified (Table V.3 and Table V.S7). Centre ants were always observed to be in the centre of the column, both on the outward and return journey (Table V.3 and Table V.7). Tail ants tended to stay at the tail but were also found in the centre of the column in subsequent outward journeys. On the return journey tail ants were either at the front or centre.

Table V.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 centre the rest of the column in between. Disproportionately large deviations from the random distribution (Front: 10%, Centre: 80%, Tail 10%) for the different categories marked in bold (see Table V.S7 for detailed statistical results). Standard deviation in brackets.

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

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; centre 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 (Table V.4). On the outward journey tail and centre ants also seemed to return to their former position. On the return journey no clear position fidelity could be observed (Table V.4), independent from the earlier position.

Table V.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 Table V.S8 for detailed statistical results).

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

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 & 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 & 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. V.1) (Longhurst & Howse 1979). In addition we now showed that the formation is even more complex, with termite carriers concentrated at the centre of the column and a rear-guard predominantly occupied by individuals best adapted to fighting off potential predators (raid-guards, Fig. V.3) (Breed & Harrison 1988, Dejean & Feneron 1996, Hölldobler & 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 & 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 (personal observation). The more vulnerable termite carriers are best protected at the centre 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 & 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 behaviour, which most likely improve their defence 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 & 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 artefact. 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 behaviour: 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 behaviour 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. V.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 & Weiser 1999), likely making them better suited as scouts. Larger ants also generally are better soldiers or guards, with stronger and larger mandibles (Breed & Harrison 1988, Dejean & Feneron 1996, Hölldobler & Wilson 1990).

The only task that seemed to be predetermined, i.e. always conducted by the same ants, was the scouting behaviour. 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 & 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 & Linsenmar 2017a), 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. V.3*B*) 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 (1990). This is also supported by studies on the polymorphic species *Neoponera laevigata* (Hölldobler & Traniello 1980) and *Centromyrmex bequaerti* (Dejean & Feneron 1996), both of which are polymorphic and specialized termite predators.

Mechanisms regulating task allocation

The mechanisms regulating division of labour and task allocation leading to collective behaviours 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 behaviours 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 & 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 labour. We agree with Jeanne (2016) that there is a clear benefit for division of labour 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 behaviour 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 behaviour of *M. analis* offers itself as a good model to study different mechanisms and factors regulating task allocation and work division in ponerine ants.

SUPPLEMENTARY MATERIAL V

Table V.S1. Statistical differences for the outward formation of the column for large ants as illustrated in Fig. V.1*A* (*ANOVA*: Column block: F_9= 22.5; P<0.001, Colony: F_5=1.82, P=0.11).

Column block	Column block	Estimate	Std. Error	*t*	Adjusted *P*-value
1-10	11-20	-20.12	4.25	-4.74	< 0.001
1-10	21-30	-33.74	4.25	-7.95	< 0.001
1-10	31-40	-36.14	4.25	-8.51	< 0.001
1-10	41-50	-32.06	4.25	-7.55	< 0.001
1-10	51-60	-34.44	4.25	-8.11	< 0.001
1-10	61-70	-26.54	4.25	-6.25	< 0.001
1-10	71-80	-25.85	4.25	-6.09	< 0.001
1-10	81-90	-15.71	4.25	-3.70	0.01
1-10	91-100	3.87	4.25	0.91	1
11-20	21-30	-13.62	4.25	-3.21	0.05
11-20	31-40	-16.02	4.25	-3.77	0.009
11-20	41-50	-11.94	4.25	-2.81	0.14
11-20	51-60	-14.32	4.25	-3.37	0.032
11-20	61-70	-6.42	4.25	-1.51	0.89
11-20	71-80	-5.73	4.25	-1.35	0.94
11-20	81-90	4.42	4.25	1.04	0.99
11-20	91-100	23.99	4.25	5.65	< 0.001
21-30	31-40	-2.40	4.25	-0.57	1
21-30	41-50	1.68	4.25	0.40	1
21-30	51-60	-0.70	4.25	-0.17	1
21-30	61-70	7.19	4.25	1.69	0.80
21-30	71-80	7.89	4.25	1.86	0.70
21-30	81-90	18.03	4.25	4.25	0.002
21-30	91-100	37.61	4.25	8.86	< 0.001
31-40	41-50	4.08	4.25	0.96	0.99
31-40	51-60	1.70	4.25	0.40	1
31-40	61-70	9.60	4.25	2.26	0.42
31-40	71-80	10.29	4.25	2.42	0.32
31-40	81-90	20.43	4.25	4.81	< 0.001
31-40	91-100	40.01	4.25	9.42	< 0.001
41-50	51-60	-2.39	4.25	-0.56	1
41-50	61-70	5.51	4.25	1.30	0.95
41-50	71-80	6.21	4.25	1.46	0.90
41-50	81-90	16.35	4.25	3.85	0.007
41-50	91-100	35.93	4.25	8.46	< 0.001
51-60	61-70	7.90	4.25	1.86	0.70
51-60	71-80	8.59	4.25	2.02	0.58
51-60	81-90	18.74	4.25	4.41	< 0.001
51-60	91-100	38.31	4.25	9.02	< 0.001
61-70	71-80	0.69	4.25	0.16	1
61-70	81-90	10.84	4.25	2.55	0.25
61-70	91-100	30.42	4.25	7.16	< 0.001
71-80	81-90	10.15	4.25	2.39	0.34
71-80	91-100	29.72	4.25	7.00	< 0.001
81-90	91-100	19.58	4.25	4.61	< 0.001

Table V.S2. Statistical differences for the return formation of the column for large ants as illustrated in Fig. V.1*B* (*ANOVA*: Column block: F_9= 22.19; P<0.001, Colony effect: F_5=2.22, P=0.056).

Column block	Column block	Estimate	Std. Error	t-value	Adjusted *P*-value
1-10	11-20	-6.94	5.61	-1.24	0.96
1-10	21-30	-16.60	5.61	-2.96	0.10
1-10	31-40	-27.86	5.61	-4.97	<0.01
1-10	41-50	-38.03	5.61	-6.78	<0.01
1-10	51-60	-46.22	5.61	-8.24	<0.01
1-10	61-70	-50.21	5.61	-8.95	<0.01
1-10	71-80	-53.17	5.61	-9.48	<0.01
1-10	81-90	-46.72	5.61	-8.33	<0.01
1-10	91-100	-32.90	5.61	-5.87	<0.01
11-20	21-30	-9.66	5.61	-1.72	0.78
11-20	31-40	-20.92	5.61	-3.73	0.01
11-20	41-50	-31.09	5.61	-5.54	<0.01
11-20	51-60	-39.28	5.61	-7.00	<0.01
11-20	61-70	-43.27	5.61	-7.71	<0.01
11-20	71-80	-46.23	5.61	-8.24	<0.01
11-20	81-90	-39.78	5.61	-7.09	<0.01
11-20	91-100	-25.96	5.61	-4.63	<0.01
21-30	31-40	-11.27	5.61	-2.01	0.59
21-30	41-50	-21.43	5.61	-3.82	<0.01
21-30	51-60	-29.62	5.61	-5.28	<0.01
21-30	61-70	-33.61	5.61	-5.99	<0.01
21-30	71-80	-36.57	5.61	-6.52	<0.01
21-30	81-90	-30.12	5.61	-5.37	<0.01
21-30	91-100	-16.30	5.61	-2.91	0.11
31-40	41-50	-10.16	5.61	-1.81	0.73
31-40	51-60	-18.35	5.61	-3.27	0.04
31-40	61-70	-22.35	5.61	-3.98	<0.01
31-40	71-80	-25.30	5.61	-4.51	<0.01
31-40	81-90	-18.86	5.61	-3.36	0.03
31-40	91-100	-5.03	5.61	-0.90	1.00
41-50	51-60	-8.19	5.61	-1.46	0.91
41-50	61-70	-12.18	5.61	-2.17	0.48
41-50	71-80	-15.14	5.61	-2.70	0.19
41-50	81-90	-8.69	5.61	-1.55	0.87
41-50	91-100	5.13	5.61	0.91	1.00
51-60	61-70	-3.99	5.61	-0.71	1.00
51-60	71-80	-6.95	5.61	-1.24	0.96
51-60	81-90	-0.50	5.61	-0.09	1
51-60	91-100	13.32	5.61	2.38	0.35
61-70	71-80	-2.96	5.61	-0.53	1
61-70	81-90	3.49	5.61	0.62	1.00
61-70	91-100	17.31	5.61	3.09	0.07
71-80	81-90	6.45	5.61	1.15	0.98
71-80	91-100	20.27	5.61	3.61	0.02
81-90	91-100	13.82	5.61	2.46	0.30

Table V.S3. Statistical differences for the return formation of a mean saturated column (<70% Termite carriers) for raid-guards as illustrated in Fig. V.4*A* (*Kruskal-Wallis*: Column block: X^2_9=32.07; *P*<0.001, Colony effect linear model: F_5=0.65, *P*=0.67).

Column block	Column block	t-value	Adjusted *P*-value
1-10	11-20	2.62	0.16
1-10	21-30	2.62	0.16
1-10	31-40	2.46	0.23
1-10	41-50	1.13	1
1-10	51-60	1.70	1
1-10	61-70	1.86	0.97
1-10	71-80	1.49	1
1-10	81-90	-0.13	0.89
1-10	91-100	-1.34	1
11-20	21-30	0.01	0.50
11-20	31-40	-0.16	1
11-20	41-50	-1.48	1
11-20	51-60	-0.92	1
11-20	61-70	-0.76	1
11-20	71-80	-1.13	1
11-20	81-90	-2.75	0.11
11-20	91-100	-3.95	0.002
21-30	31-40	-0.17	1
21-30	41-50	-1.49	1
21-30	51-60	-0.93	1
21-30	61-70	-0.76	1
21-30	71-80	-1.13	1
21-30	81-90	-2.76	0.11
21-30	91-100	-3.96	0.002
31-40	41-50	-1.32	1
31-40	51-60	-0.76	1
31-40	61-70	-0.59	1
31-40	71-80	-0.96	1
31-40	81-90	-2.59	0.17
31-40	91-100	-3.79	0.003
41-50	51-60	0.57	1
41-50	61-70	0.73	1
41-50	71-80	0.36	1
41-50	81-90	-1.27	1
41-50	91-100	-2.47	0.23
51-60	61-70	0.16	1
51-60	71-80	-0.21	1
51-60	81-90	-1.83	1
51-60	91-100	-3.04	0.049
61-70	71-80	-0.37	1
61-70	81-90	-2.00	0.73
61-70	91-100	-3.20	0.03
71-80	81-90	-1.63	1
71-80	91-100	-2.83	0.09
81-90	91-100	-1.20	1

Table V.S4. Statistical differences for the return formation of a mean saturated column (<70% termite carriers) for termite carriers as illustrated in Fig. V.4*A* (*Kruskal Wallis*: Column block: $X^2_9=74.42$; $P<0.001$; Colony effect linear model: $F_5=1.18$, $P=0.33$).

Column block	Column block	t-value	Adjusted *P*-value
1-10	11-20	-0.81	1
1-10	21-30	-0.34	1
1-10	31-40	0.80	1
1-10	41-50	2.17	0.34
1-10	51-60	2.74	0.08
1-10	61-70	3.71	0.004
1-10	71-80	3.83	0.002
1-10	81-90	4.27	<0.001
1-10	91-100	4.55	<0.001
11-20	21-30	0.46	1
11-20	31-40	1.60	0.98
11-20	41-50	2.98	0.04
11-20	51-60	3.55	0.006
11-20	61-70	4.51	<0.001
11-20	71-80	4.64	<0.001
11-20	81-90	5.08	<0.001
11-20	91-100	5.36	<0.001
21-30	31-40	1.14	1
21-30	41-50	2.51	0.15
21-30	51-60	3.08	0.03
21-30	61-70	4.05	<0.001
21-30	71-80	4.17	<0.001
21-30	81-90	4.61	<0.001
21-30	91-100	4.89	<0.001
31-40	41-50	1.37	1
31-40	51-60	1.94	0.55
31-40	61-70	2.91	0.04
31-40	71-80	3.03	0.03
31-40	81-90	3.47	0.008
31-40	91-100	3.75	0.003
41-50	51-60	0.57	1
41-50	61-70	1.54	1
41-50	71-80	1.66	0.92
41-50	81-90	2.10	0.39
41-50	91-100	2.38	0.21
51-60	61-70	0.97	1
51-60	71-80	1.09	1
51-60	81-90	1.53	1
51-60	91-100	1.81	0.71
61-70	71-80	0.12	0.45
61-70	81-90	0.56	1
61-70	91-100	0.84	1
71-80	81-90	0.44	1
71-80	91-100	0.72	1
81-90	91-100	0.28	0.78

Table V.S5. Statistical differences for the return formation of an oversaturated column (>70% termite carriers) for raid-guards as illustrated in Fig. V.4*B* (*ANOVA*: Column block: F_9= 5.23; *P*<0.001, Colony effect: F_5=0.84, *P*=0.53).

Column block	Column block	Estimate	Std. Error	t-value	Adjusted *P*-value
1-10	11-20	-29.60	6.51	-4.55	0.003
1-10	21-30	-25.58	6.51	-3.93	0.015
1-10	31-40	-25.66	6.51	-3.94	0.014
1-10	41-50	-29.10	6.51	-4.47	0.004
1-10	51-60	-27.54	6.51	-4.23	0.007
1-10	61-70	-26.94	6.51	-4.14	0.008
1-10	71-80	-30.14	6.51	-4.63	0.003
1-10	81-90	-27.25	6.51	-4.19	0.008
1-10	91-100	-6.60	6.51	-1.02	0.99
11-20	21-30	4.02	6.51	0.62	1
11-20	31-40	3.95	6.51	0.61	1
11-20	41-50	0.50	6.51	0.08	1
11-20	51-60	2.07	6.51	0.32	1
11-20	61-70	2.66	6.51	0.41	1
11-20	71-80	-0.53	6.51	-0.08	1
11-20	81-90	2.35	6.51	0.36	1
11-20	91-100	23.00	6.51	3.53	0.04
21-30	31-40	-0.07	6.51	-0.01	1
21-30	41-50	-3.52	6.51	-0.54	1
21-30	51-60	-1.95	6.51	-0.30	1
21-30	61-70	-1.36	6.51	-0.21	1
21-30	71-80	-4.55	6.51	-0.70	1
21-30	81-90	-1.67	6.51	-0.26	1
21-30	91-100	18.98	6.51	2.92	0.15
31-40	41-50	-3.45	6.51	-0.53	1
31-40	51-60	-1.88	6.51	-0.29	1
31-40	61-70	-1.28	6.51	-0.20	1
31-40	71-80	-4.48	6.51	-0.69	1
31-40	81-90	-1.60	6.51	-0.25	1
31-40	91-100	19.05	6.51	2.93	0.14
41-50	51-60	1.57	6.51	0.24	1
41-50	61-70	2.16	6.51	0.33	1
41-50	71-80	-1.03	6.51	-0.16	1
41-50	81-90	1.85	6.51	0.28	1
41-50	91-100	22.50	6.51	3.46	0.045
51-60	61-70	0.60	6.51	0.09	1
51-60	71-80	-2.60	6.51	-0.40	1
51-60	81-90	0.28	6.51	0.04	1
51-60	91-100	20.93	6.51	3.22	0.08
61-70	71-80	-3.20	6.51	-0.49	1
61-70	81-90	-0.31	6.51	-0.05	1
61-70	91-100	20.34	6.51	3.12	0.095
71-80	81-90	2.88	6.51	0.44	1
71-80	91-100	23.53	6.51	3.62	0.03
81-90	91-100	20.65	6.51	3.17	0.08

Table V.S6. Statistical differences for the return formation of an oversaturated column (>70% termite carriers) for termite carriers as illustrated in Fig. V.4*B* (*ANOVA*: Column block: F_9= 4.5; P<0.001; Colony effect: F_5=1.1, P=0.39).

Column block	Column block	Estimate	Std. Error	t-value	Adjusted *P*-value
1-10	11-20	21.29	9.49	2.24	0.45
1-10	21-30	6.62	9.49	0.70	1
1-10	31-40	8.11	9.49	0.85	1
1-10	41-50	7.90	9.49	0.83	1
1-10	51-60	1.86	9.49	0.20	1
1-10	61-70	0.95	9.49	0.10	1
1-10	71-80	-11.60	9.49	-1.22	0.96
1-10	81-90	-22.12	9.49	-2.33	0.40
1-10	91-100	-24.15	9.49	-2.54	0.29
11-20	21-30	-14.67	9.49	-1.55	0.86
11-20	31-40	-13.18	9.49	-1.39	0.92
11-20	41-50	-13.39	9.49	-1.41	0.91
11-20	51-60	-19.42	9.49	-2.05	0.58
11-20	61-70	-20.34	9.49	-2.14	0.51
11-20	71-80	-32.88	9.49	-3.46	0.045
11-20	81-90	-43.40	9.49	-4.57	<0.01
11-20	91-100	-45.44	9.49	-4.79	<0.01
21-30	31-40	1.49	9.49	0.16	1
21-30	41-50	1.28	9.49	0.14	1
21-30	51-60	-4.75	9.49	-0.50	1
21-30	61-70	-5.67	9.49	-0.60	1
21-30	71-80	-18.21	9.49	-1.92	0.66
21-30	81-90	-28.73	9.49	-3.03	0.12
21-30	91-100	-30.77	9.49	-3.24	0.07
31-40	41-50	-0.21	9.49	-0.02	1
31-40	51-60	-6.25	9.49	-0.66	1
31-40	61-70	-7.16	9.49	-0.75	1
31-40	71-80	-19.71	9.49	-2.08	0.56
31-40	81-90	-30.23	9.49	-3.18	0.08
31-40	91-100	-32.26	9.49	-3.40	0.051
41-50	51-60	-6.04	9.49	-0.64	1
41-50	61-70	-6.95	9.49	-0.73	1
41-50	71-80	-19.49	9.49	-2.05	0.57
41-50	81-90	-30.02	9.49	-3.16	0.09
41-50	91-100	-32.05	9.49	-3.38	0.054
51-60	61-70	-0.91	9.49	-0.10	1
51-60	71-80	-13.46	9.49	-1.42	0.91
51-60	81-90	-23.98	9.49	-2.53	0.30
51-60	91-100	-26.01	9.49	-2.74	0.20
61-70	71-80	-12.55	9.49	-1.32	0.94
61-70	81-90	-23.07	9.49	-2.43	0.34
61-70	91-100	-25.10	9.49	-2.64	0.24
71-80	81-90	-10.52	9.49	-1.11	0.98
71-80	91-100	-12.56	9.49	-1.32	0.94
81-90	91-100	-2.03	9.49	-0.21	1

Table V.S7. Statistical differences for position fidelity in different raids for the outward and return journey as illustrated in Table V.3 (Kolmogorov-Smirnov Test with Bonferroni correction, values were tested against a group with random distribution within the column).

Column block	Position of marked ants	*D*	Adjusted *P*-value	Number of raids (*N*)
Outward Journey				
Front	Front	0.94	<0.001	18
Front	Centre	0.78	<0.001	18
Front	Tail	0.56	0.02	18
Centre	Front	0.54	0.14	13
Centre	Centre	0.15	1	13
Centre	Tail	0.54	0.87	13
Tail	Front	0.82	0.004	11
Tail	Centre	0.45	0.6	11
Tail	Tail	0.55	0.23	11
Return Journey				
Front	Front	0.57	0.6	7
Front	Centre	0.86	0.03	7
Front	Tail	0.71	0.17	7
Centre	Front	0.63	0.26	8
Centre	Centre	0.38	1	8
Centre	Tail	1	<0.001	8
Tail	Front	0.67	1	5
Tail	Centre	0.67	1	5
Tail	Centre	1	0.04	5

Table V.S8. Statistical differences for position fidelity in the same raid for the outward journey as illustrated in Table V.4 (Fisher's exact test with Bonferroni correction, values were tested against a group that would show no fidelity).

Position Fidelity	Direction	Adjusted *P*-value	Number of raids (*N*)
Front	Outward	<0.001	10
Centre	Outward	0.02	10
Tail	Outward	0.004	10
Front	Return	1	10
Centre	Return	1	10
Tail	Return	1	10

Successful *Megaponera analis* raid preparing for the return journey after collecting their termite prey

CHAPTER VI: GENERAL DISCUSSION

In my thesis I extensively studied the foraging behaviour of *M. analis*, with the aim to better understand how group-raiding ants specialized on a defensive prey have adapted their foraging behaviour to maximize their net energy intake. These adaptations addressed the problems of having to cope with an increased injury risk in the work force (Chapter II and III). The necessity to find constantly changing food sources (both in quality and location) in a heterogeneous environment and passing that information to the colony (Chapter III). Furthermore, predation risks were minimized through a column formation and a large flexibility in task allocations during the hunt enabled the maximization of profits in a very short time window (Chapter IV).

Rescue behaviour

In chapter II the value of a rescue behaviour in a social predator focused on highly defensive prey was shown. Remarkably this behaviour was focused not only on handicapped ants (clinging termites) but also on nestmates that suffered severe injuries in the form of lost extremities. Furthermore, the gland and the compounds responsible for the "call for help" were identified. Lastly a model for quantifying the value of this behaviour for the colony was proposed. According to this model, the presence of the rescue behaviour allows the colony to be 29% larger than one that would not rescue their injured nestmates.

Rescue behaviour has been previously observed in ants (Hollis & Nowbahari 2013a). Ants that are trapped under soil after a cave in are dug out by nestmates (Hangartner 1969). Ants caught by an antlion are pulled out/rescued by nestmates (Nowbahari et al. 2009). However, one big difference to the present behaviour is that in these latter situations the ants are directly confronted with an imminent danger: suffocating or being eaten. This is not the case in *M. analis*: not only are the injured ants often handicapped for life through the loss of extremities, but the danger towards these ants is also far less obvious. These ants are not under a direct threat but rather under an abstract increased predation risk if they were to return alone.

The rescue behaviour in *M. analis* does have certain antecedents in ants. The closely related species *Paltothyreus tarsatus* rescues ants trapped under soil using the same distress pheromones as *M. analis* (DMDS and DMTS) from their mandibular gland (Crewe & Fletcher 1974). Carrying nestmates is widespread in ants for various different reasons

(Hölldobler & Wilson 1990). *Megaponera analis* uses carrying behaviour not only to save injured ants but also to transport juveniles during emigrations and to dispose of dead nestmates (personal observation). For this behaviour to be used also in the context of rescue behaviour should not require many evolutionary steps. I explore the evolution and mechanisms of rescue behaviour further in Frank & Linsenmair 2017c.

In chapter III I further show that this behaviour is only concentrated towards lightly injured ants (loss of less than two limbs), while heavily injured ants (loss of five limbs) are not helped. Interestingly this behaviour was not regulated by the helper but unconsciously by the injured ant itself; if it was unable to return to a resting position it did not call for help. Thus leading to a helping triage that is regulated from the bottom up, unlike in humans were the helper makes the decision (Widgren & Jourak 2011). This phenomenon exemplifies the importance of inclusive fitness in the behavioural responses of ants and underscores the argument that empathy does not play a part in this rescue behaviour (Vasconcelos et al. 2012).

My results are the first to explore the adaptive value of this form of rescue behaviour focused on injured nestmates in social insects and help us identify evolutionary drivers responsible for this type of behaviour to evolve. It shows the importance individuals play in ant colonies, thereby challenging the general view of the individual ant in the work force as disposable. Furthermore, I demonstrate that complex rescue behaviour can evolve in unique situations if the necessary drivers are present, even in species that are very likely unable to recognize the increased risk to which they are exposed to themselves.

The Pan-African distribution of *M. analis* allows for very promising comparative studies in which injury risks and colony sizes differ. The proposed model further allows us to identify other potential species in which this behaviour is likely to be present. For instance, the group hunting termite specialist *Leptogenys* sp. also has relatively small colony sizes (potentially high value of individuals) and hunts termites in groups (social hunter and a defensive prey) (Ganeshaiah & Veena 1991). It is still debated if empathy is necessary for pro-social behaviour to occur in animals (Vasconcelos et al. 2012, Hollis & Nowbahari 2013a). In our study I argue that the same problem has arisen in many different animal orders (how to rescue injured members of the group to indirectly or directly increase the fitness of the helper). This led to the evolution of different mechanisms that deal with the same problem. While humans and potentially other mammals and birds use empathy (Vasconcelos et al. 2012, Decety et al. 2016, Hammers & Brouwer 2017), ants use chemical communication. The different selection pressures prevailing in different ecosystems and species could therefore allow us to get a better understanding what the driving factors for the evolution and emergence of rescue behaviour are.

Social Immunity

In chapter III I investigated the health hazard open wounds pose to injured individuals and how these risks are reduced. I show for the first time treatment of open wounds by other individuals in social insects, which reduces mortality from 80% to 10%.

Social immunity allows ants to compensate the increased infection risks of living in high concentrations in a confined space with limited genetic diversity (Cremer et al. 2007). The topic has gained increased attention in the last decade due to the implications it has on infection outbreaks in large societies (Cremer et al. 2007), with applications also for human populations (i.e. how to quarantine a city etc.). Ants offer themselves as ideal model organisms to study these questions due to the feasibility of experiments in the lab and the complex interaction networks they have (Hölldobler & Wilson 2008, Cremer et al. 2007). The queen is the most valuable member of the colony (Hölldobler & Wilson 1990), interaction networks between workers therefore limit contacts to the queen to the ants responsible for brood care, which are therefore less likely to carry an infection (Cremer et al. 2007). This reduces infection risk of the most valuable members of the colony. At the same time ants that were parasitized by fungi receive increased allogrooming treatment by nestmates to improve immunization of the colony by getting in contact with the pathogen (Ugelvig & Cremer 2007). Foragers are also generally more present at the entrance of the nest, thereby reducing the probability of an infection outbreak in the colony (Cremer et al. 2007).

It was therefore interesting to see how ants reacted to injured individuals that lost extremities. Open wounds are a major health hazard in animals by drastically increasing the risk of infection (Siva-Jothy et al. 2005). The novel mechanism I described, of intense allogrooming at the wound by nestmates, is able to reduce mortality significantly. Moreover the very fast wound sealing by individuals without treatment (within ten minutes), shows also clear adaptations to reduce the costs of increased injury risks on the individual level. This combination of a social and individual level response raises various interesting follow up questions: How do the ants know which individuals need treatment? What does the treatment exactly do? Excretions of the metapleural and venom gland are known to harbour antibacterial substances (Fernandez-Marin et al. 2006, Tragust 2016), potentially the mandibular gland of *M. analis* also harbours such compounds which are applied on the wound. What is the benefit of rapid wound closure if the treatment occurs after the closing of the wound? How important is the timing of the treatment? Does it have to occur within the first hour after injury or can it occur at a later time? Is there an infection risk trade-off for the treatment? I.e. when the infection is too advanced treatment could pose a too great risk for the helper. Answering these follow up questions can allow us to reach a better understanding of insect immunity both on the social and individual level and could hold valuable insights towards the study of epidemiology.

Decision-making and optimal foraging

In Chapter IV I show that optimal foraging theory can adequately predict most of the foraging behaviour observed in *M. analis*. Collective decision-making is one of the main mechanisms of organization in social insects (Hölldobler & Wilson 2008). It was therefore promising to study how a species in which only a hand full of individuals (the scouts) held all the information about the foraging sites regulated their decisions in line with optimal foraging theory. Remarkably, most of the foraging decisions in *M. analis* were not achieved through collective decisions but rather by individual decisions of scout ants. Thus, 1% of the colony (10-20 scouts) decided the fate and foraging efficiency of the remaining 99%.

This raised various interesting questions towards the cost and benefits of individual decisions when compared to collective ones and when it is appropriate to use which strategy. Collective decisions are less likely to lead to mistakes, although this comes at the cost of time (Chittka et al. 2009). When rapid decisions are necessary individual decisions therefore are beneficial, for instance when hunting a mobile prey (Witte et al. 2010). When the cost of a mistake becomes too large a collective decision might be better. In *M. analis* this is the case during emigrations. While it is also only one scout that at first finds a new nest site, the decision is not made by this one individual. Instead the scout recruits 10-20 former scouts to the new nest and each scout individually returns to the nest to recruit more nestmates until a certain quorum threshold is reached and the emigration starts (Frank, Fischer, Linsenmair in preparation).

In ants it is generally believed that most of the behaviours are regulated collectively (Hölldobler & Wilson 2008). In this study I show that the importance of individual scouts and experience cannot be underestimated. The theory that division of labour increases the efficiency of individuals when they perform specialised tasks is still disputed (Chittka and Muller 2009). The importance of experience in decision-making in ants is also still debated (Ravary et al. 2007, Robinson et al. 2012). The age/experience trade-off is especially interesting. In general older ants perform riskier tasks, scouts for instance are generally the oldest ants (Hölldobler & Wilson 2008). At the same time older ants are also more experienced and therefore could become more valuable in species in which experience is more important for the foraging success. This is also exemplified in the path choice to the raiding site in *M. analis*. Instead of walking the shortest way back to the nest scouts are able to walk the fastest way back (Hönle, Frank, Linsenmair submitted). In a heterogeneous environment were different substrates lead to different running speeds the necessary calculations for the fastest path become increasingly complex. Other ant species generally regulate path choice through collective decisions: pheromone trail reinforcement (Hölldobler & Wilson 2008), how *M. analis* scouts are able to do it individually is still unknown, it very likely requires a large amount of experience about the surrounding area. A more in depth look at the scouting behaviour of *M. analis* could help answer these questions.

The fact that scouts are able to regulate the number of nestmates participating in raids depending on the foraging site raises further questions. How does recruitment work / how does the scout convey this information? How does the scout investigate a food source to adequately estimate its value? How is the hunger state of the colony conveyed to the scout? Further studies could help us better understand the value of experience in ants and how the proportion of scouts in a social animal should vary depending on the ecological environment and natural history of the species.

Task allocation and formation

In Chapter V I show new tasks and formation patterns within the raiding behaviour of *M. analis*. Interestingly, most of these tasks seemed not to be predetermined before the raid but were filled out as need arose during the raid. The column formation was also more sophisticated than previously thought. Larger ants acted as van- and rear-guard and were present in larger numbers at these positions. The formation was also far more stable, with individuals assuming the same position over subsequent raids and front ants even returning to this position if displaced within the same raid.

Formations within raiding columns have rarely been studied, even though they are an integral part of the foraging process (roughly 2/3 of the foraging time is spend travelling in *M. analis*). The more selection pressures are present during this travel time the more likely it is to become more sophisticated. The increased injury risk led to two new roles of lingerers and runners looking for injured ants to pick up and carry back. The van- and rear-guard likely improve defensive capabilities of the column against predators. These are all adaptations that reduce the costs involved in the foraging process of *M. analis*. The work division at the hunting ground further improves the capabilities of killing as many termites as possible before they escape/retreat. The necessity to maximize time efficiency when hunting termites likely also led to the unique continuous allometric size polymorphism in this ponerine ant (Villet 1990). Thus allowing greater flexibility in task allocation, a necessity due to the unpredictability of task requirements in an irregular system such as the group-hunting of termites.

This raises various interesting questions on the underlying mechanisms regulating the formation, work division and task allocation. How is the formation within the column regulated and how do individual ants know if they have been displaced from their position? First results seem to suggest that the raidleader might emit certain short chained polar substances for a temporal individual recognition by nestmates, which could help them find the front of the column (Frank & Schmitt in preparation). Why are former scouts concentrated at the front behind the raid leader? Do all ants go through the different foraging roles in their lifetime or are other factors vital? The unique foraging behaviour of *M. analis* therefore offers itself as a good model organism to further study the different mechanisms and factors regulating task allocation and work division in ants. This could help us create better models on how work division should be regulated when the necessity of tasks is unknown prior to deployment.

SYNTHESIS

I have shown that specialization towards a highly defensive prey can lead to very unique adaptations in the foraging behaviour of a species. All of my observations help us to further understand how a group-hunting predator should forage so as to minimize costs while maximizing profits. To minimize costs injured individuals should be rescued and treated back to health, as long as the individual is useful to the group after recovery. The raid column should have a formation to minimize costs of predation risks and increase the likelihood of finding injured individuals. To maximize net energy gain group size should be adapted to the foraging site. Worker sizes should be distributed in a way that allows large flexibility, which is necessary when hunting prey for which task requirements are unknown prior to the hunt. There are still a lot of open questions left, mentioned throughout the discussion, and addressing them will lead to greater insights in optimal foraging theory and how it applies to group hunting predators.

A jumping spider (Salticidae) hunting a *Megaponera analis* worker

REFERENCES

Abou-Shaara HF (2014). The foraging behaviour of honey bees, *Apis mellifera*: a review. *Veterinarni Medicina* 59(1):1–10

Agosti D, Johnson NF (2003). La nueva taxonomía de hormigas. In Fernández F (ed.). Introducción a las Hormigas de la region Neotropical (pp.45–48). Instituto de Investigación de Recursos Biológicos Alexander von Humboldt. Bogotá, Colombia

Anderson C, Boomsma JJ, Bartholdi JJ (2002). Task partitioning in insect societies: bucket brigades. *Insectes Sociaux* 49:171–180

Bailey KH, Polis GA (1987). Optimal and central-place foraging theory applied to a desert harvester ant, *Pogonomyrmex californicus*. *Oecologia* 72:440–448

Bartal IBA, Decety J, Mason P (2011). Empathy and Pro-Social Behavior in Rats. *Science* 334:1427–1430

Bayliss J, Fielding A (2002). Termitophagous foraging by *Pachycondyla analis* (Formicidae, Ponerinae) in a Tanzanian coastal dry forest. *Sociobiology* 39:103–122

Beck J, Kunz BK (2007). Cooperative self-defence: Matabele ants (*Pachycondyla analis*) against African driver ants (*Dorylus* sp.; Hymenoptera: Formicidae). *Myrmecological News* 10:27–28

Bignell DE, Roisin Y, Lo N (eds.) (2011). Biology of Termites: a Modern Synthesis. Springer Verlag. Berlin, Germany.

Bonabeau E, Theraulaz G, Deneubourg JL (1998). Fixed response tresholds and the regulation of division of labor in insect societies. *Bulletin of Mathematical Biology* 60:753–807

Bonser R, Wright PJ, Bament S, Chukwu UO (1998). Optimal patch use by foraging workers of *Lasius fuliginosus*, *L. niger* and *Myrmica ruginodis*. *Ecological Entomology* 23(1):15–21

Breed MD, Harrison JM (1988). Worker Size, Ovary Development and Division of Labor in the Giant Tropical Ant, *Paraponera clavata* (Hymenoptera, Formicidae). *Journal of the Kansas Entomological Society* 61:285–291

Burgeon L (1929). Une organisation sanitaire chez les fourmis *Megaponera*. *Revue de Zoologie et de Botanique Africaines* 16:94–95.

Cartar RV, Dill LM (1990). Why Are Bumble Bees Risk-Sensitive Foragers. *Behavioral Ecology and Sociobiology* 26(2):121–127.

Castella G, Chapuisat M, Christe P (2008). Prophylaxis with resin in wood ants. *Animal Behaviour* 75:1591–1596

Cerdá X (1988). Food collection by *Cataglyphis iberica* (EM.) (Hymenoptera, Formicidae). *Annales Zoologici* 18:515–525

Chapuisat M (2010). Social Evolution: Sick Ants Face Death Alone. *Current Biology* 20(3):104-105

Charnov EL (1976). Optimal Foraging, Marginal Value Theorem. *Theoretical Population Biology* 9(2):129–136

Chittka L, Muller H (2009). Learning, specialization, efficiency and task allocation in social insects. Communicative & Integrative Biology 2(2):151–154

Chittka L, Skorupski P, Raine NE (2009). Speed-accuracy tradeoffs in animal decision making. *Trends in Ecology & Evolution* 24(7):400–407

Chouvenc T, Efstathion CA, Elliott ML, Su NY (2013). Extended disease resistance emerging from the faecal nest of a subterranean termite. *Proceedings of the Royal Society B* 280:1770

Connétable S, Robert A, Bouffault F, Bordereau C (1999). Vibratory Alarm Signals in Two Sympatric Higher Termite Species: *Pseudocanthotermes spiniger* and *P. militaris* (Termitidae, Macrotermitinae). *Journal of Insect Behavior* 12(3):329–342

Corbara B, Dejean A (2000). Adaptive behavioral flexibility of the ant *Pachycondyla analis* (=*Megaponera foetens*) (Formicidae : Ponerinae) during prey capture. *Sociobiology* 36:465–483

Cremer S, Armitage SAO, Schmid-Hempel P (2007). Social immunity. *Current Biology* 17(16):693–702

Crewe RM, Fletcher DJC (1974). Ponerine ant secretions: The mandibular gland secretion of *Paltothyreus tarsatus* Fabr. *Journal of the Entomological Society of Southern Africa* 37:291–298

Crewe RM, Peeters CP, Villet M (1984). Frequency-distribution of worker sizes in *Megaponera foetens* (Fabricius). *South African Journal of Zoology* 19(3):247–248

De Roode JC, Lefevre T, Hunter MD (2013). Self-Medication in Animals. *Science* 340(6129):150–151

Dawkins R, Krebs JR (1979). Arms races between and within species. *Proceedings of the Royal Society B* 205:489–511

Decety J, Bartal IB, Uzefovsky F, Knafo-Noam A (2016) Empathy as a driver of prosocial behaviour: highly conserved neurobehavioural mechanisms across species. *Philosophical transactions of the Royal Society B* 371:20150077

Dejean A, Feneron R (1996). Polymorphism and oligogyny in the ponerine ant *Centromyrmex bequaerti* (Formicidae:Ponerinae). *Insectes Sociaux* 43:87–99

Dornhaus A, Chittka L (2004a). Information flow and regulation of foraging activity in bumble bees (*Bombus* spp.). *Apidologie* 35:183–192

Dornhaus A, Chittka L (2004b). Why do honey bees dance? *Behavioural Ecology and Sociobiology* 55:395–401

Fernandez-Marin H, Zimmerman JK, Rehner SA, Wcislo WT (2006). Active use of the metapleural glands by ants in controlling fungal infection. *Proceedings of the Royal Society B* 273(1594):1689–1695

Frank ET, Linsenmair KE (2017a). Individual versus collective decision making: optimal foraging in the group hunting termite specialist *Megaponera analis*. *Animal Behaviour* 130:27–35

Frank ET, Linsenmair KE (2017b). Flexible task allocation and raid organization in the termite-hunting ant *Megaponera analis*. *Insectes Sociaux,* Online first doi: 10.1007/s00040-017-0579-2

Frank ET, Linsenmair KE (2017c) Saving the injured: convergent evolution and mechanisms. *Communicative and Integrative Biology*, in press

Frank ET, Schmitt T, Hovestadt T, Mitesser O, Stiegler J, Linsenmair KE (2017). Saving the injured: rescue behavior in the termite hunting ant *Megaponera analis*. *Science Advances* 3:e1602187

Ganeshaiah KN, Veena T (1991). Topology of the Foraging Trails of *Leptogenys-Processionalis* - Why Are They Branched. *Behavioral Ecology and Sociobiology* 29:263–270

Gill FB (2006). Ornithology 3rd Edition. W.H. Freeman, London, UK

Gordon DM (2016). From division of labor to the collective behavior of social insects. *Behavioral Ecology and Sociobiology* 70:1101–1108

Gruter C, von Zuben LG, Segers FHID, Cunningham JP (2016). Warfare in stingless bees. *Insectes Sociaux* 63(2):223–236

Hammers M, Brouwer L (2017). Rescue behaviour in a social bird: removal of sticky “bird-catcher tree” seeds by group members. *Behaviour* 154(4):403–411

Hangartner W (1969). Carbon dioxide, a releaser for digging behavior in *Solenopsis geminata* (Hymenoptera: Formicidae). *Psyche* 76:58–67

Heinze J, Walter B (2010). Moribund Ants Leave Their Nests to Die in Social Isolation. *Current Biology* 20(3):249–252

Herb BR, Wolschin F, Hansen K, Aryee MJ, Langmead B, Irizarry R, Amdam GV, Feinberg AP (2012). Reversible switching between epigenetic states in honeybee behavioral subcastes. *Nature Neuroscience* 15:1371–1373

Hölldobler B, Braun U, Gronenberg W, Kirchner WH, Peeters C (1994). Trail communication in the ant *Megaponera foetens* (Fabr.) (Formicidae, Ponerinae). *Journal of Insect Physiology* 40:585–593

Hölldobler B, Traniello JFA (1980). The Pygidial Gland and Chemical Recruitment Communication in *Pachycondyla* (= *Termitopone*) *laevigata*. *Journal of Chemical Ecology* 6:883–893

Hölldobler B, Wilson EO (1990). The Ants. Belknap Press of Harvard University Press, Cambridge, MA

Hölldobler B, Wilson EO (2008). The Superorganism. W.W. Norton & Company, New York, NY

Hollis KL, Harrsch FA, Nowbahari E (2015). Ants vs. antlions: An insect model for studying the role of learned and hard-wired behavior in coevolution. *Learning and Motivation* 50:68–82

Hollis KL, Nowbahari E (2013a). Toward a Behavioral Ecology of Rescue Behavior. *Evolutionary Psychology* 11:647–664

Hollis KL, Nowbahari E (2013b). A comparative analysis of precision rescue behaviour in sand-dwelling ants. *Animal Behaviour* 85:537–544

Howard DF, Tschinkel WR (1976). Aspects of Necrophoric Behavior in Red Imported Fire Ant, *Solenopsis-Invicta*. *Behaviour* 56:157–180

Huffman MA (2003). Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. *Proceedings of the Nutrition Society* 62(2):371–381

Hughes WOH, Eilenberg J, Boomsma JJ (2002). Trade-offs in group living: transmission and disease resistance in leaf-cutting ants. *Proceedings of the Royal Society B: Biological Sciences* 269(1502):1811–1819

Jeanne RL (2016). Division of labor is not a process or a misleading concept. *Behavioral Ecology and Sociobiology* 70(7):1109–1112

Kaspari M, Weiser MD (1999). The size-grain hypothesis and interspecific scaling in ants. *Functional Ecology* 13:530–538.

Konaté S, Kampmann D (2010). Biodiversity atlas of West Africa, Volume 3: Côte d'Ivoire. BIOTA 2010, Abidjan/Frankfurt am Main.

Korczyńska J, Szczuka A, Symonowicz B, Wnuk A, Anna GS, Mazurkiewicz PJ, Studnicki M, Godzińska EJ (2014). The effects of age and past and present behavioral specialization on behavior of workers of the red wood ant *Formica Polyctena* Först. during nestmate reunion tests. *Behavioural Processes* 107:29–41

Leclerc JB, Detrain C (2017). Loss of attraction for social cues leads to fungal-infected *Myrmica rubra* ants withdrawing from the nest. *Animal Behaviour* 129:133–141

Lefevre T, Oliver L, Hunter MD, de Roode JC (2010). Evidence for trans-generational medication in nature. *Ecology Letters* 13(12):1485–1493

Lepage MG (1981). Étude de la prédation de *Megaponera foetens* (F.) sur les populations récoltantes de Macrotermitinae dans un ecosystème semi-aride (Kajiado-Kenya). *Insectes Sociaux* 28(3):247–262

Levieux J (1966). Note préliminaire sur les colonnes de chasse de Megaponera fœtens F. (Hyménoptère Formicidæ). *Insectes Sociaux* 13(2):117–126

Liebig J, Peeters C, Hölldobler B (1999). Worker policing limits the number of reproductives in a ponerine ant. *Proceedings of the Royal Society of London B* 266:1865–1870

Longhurst C, Baker R, Howse PE (1979). Termite predation by *Megaponera foetens* (FAB.) (Hymenoptera: Formicidae). *Journal of Chemical Ecology* 5:703–719

Longhurst C, Howse PE (1979). Foraging, recruitment and emigration in *Megaponera foetens* (Fab.) (Hymenoptera: Formicidae) from the Nigerian Guinea Savanna. *Insectes Sociaux* 26:204–215

Longhurst C, Johnson RA, Wood TG (1978). Termite Predation by *Megaponera foetens* (Fabr) (Hymenoptera - Formicidae) on termites in Nigerian southern Guinea savanna. *Oecologia* 32:101–107

MacArthur RH, Pianka ER (1966). On Optimal Use of a Patchy Environment. *American Naturalist* 100(916):603–609

Mallon EB, Pratt SC, Franks NR (2001). Individual and collective decision-making during nest site selection by the ant *Leptothorax albipennis*. *Behavioral Ecology and Sociobiology* 50(4):352–359

Meunier J (2015). Social immunity and the evolution of group living in insects. *Philosophical Transactions of the Royal Society of London B* 370:20140102

Milan NF, Kacsoh BZ, Schlenke TA (2012). Alcohol Consumption as Self-Medication against Blood-Borne Parasites in the Fruit Fly. *Current Biology* 22(6):488–493

Miler K (2016). Moribund Ants Do Not Call for Help. *PloS One* 11(3):e0151925

Molet M, Chittka L, Stelzer RJ, Streit S, Raine NE (2008). Colony nutritional status modulates worker responses to foraging recruitment pheromone in the bumblebee *Bombus terrestris*. *Behavioral Ecology and Sociobiology* 62:1919-1926

Moron D, Lenda M, Skorka P, Woyciechowski M (2012). Short-Lived Ants Take Greater Risks during Food Collection. *American Naturalist* 180(6):744–750

Moron D, Witek M, Woyciechowski M (2008) Division of labour among workers with different life expectancy in the ant *Myrmica scabrinodis*. *Animal Behaviour* 75:345–350

Naug D (2016). From division of labor to collective behavior: behavioral analyses at different levels. *Behavioral Ecology and Sociobiology* 70:1113–1115

Nieh, JC (2004). Recruitment communication in stingless bees (Hymenoptera, Apidae, Meliponini). *Apidologie* 35(2):159–182

Nowbahari E, Scohier A, Durand JL, Hollis KL (2009). Ants, *Cataglyphis cursor*, Use Precisely Directed Rescue Behavior to Free Entrapped Relatives. *PloS One* 4:e6573

Oi DH, Pereira RM (1993). Ant Behavior and Microbial Pathogens (Hymenoptera, Formicidae). *Florida Entomologist* 76(1):63–74

Olsson O, Brown JS, Helf KL (2008). A guide to central place effects in foraging. *Theoretical Population Biology* 74(1):22–33

Orians GH, Pearson NE (1979). On the theory of central place foraging. In D. J. Horn, R. D. Mitchell & G. R. Stairs (Eds.), Analysis of Ecological Systems (pp. 154-177). Ohio State University Press, Columbus OH

Pearce-Duvet JMC, Elemans CPH, Feener DH (2011). Walking the line: search behavior and foraging success in ant species. *Behavioral Ecology* 22(3):501–509

Peeters C, Crewe R (1987). Foraging and Recruitment in Ponerine Ants: Solitary Hunting in the Queenless *Ophthalmopone berthoudi* (Hymenoptera: Formicidae). *Psyche* 94(2):201–214

Peeters C, De Greef S (2015). Predation on large millipedes and self-assembling chains in *Leptogenys* ants from Cambodia. *Insectes Sociaux* 62:471–477

Pohl S, Foitzik S (2011). Slave-making ants prefer larger, better defended host colonies. *Animal Behaviour* 81(1):61–68

Prestwich GD (1984). Defense-mechanisms of termites. *Annual Review of Entomology* 29:201–232

Pritchard DJ (2016). Grooming by honey bees as a component of *varroa* resistant behavior. *Journal of Apicultural Research* 55(1):38–48

Raimundo RLG, Freitas AVL, Oliveira PS (2009). Seasonal Patterns in Activity Rhythm and Foraging Ecology in the Neotropical Forest-Dwelling Ant, *Odontomachus chelifer* (Formicidae:Ponerinae). *Entomological Society of America* 102(6):1151–1157

Ravary F, Lecoutey E, Kaminski G, Châline N, Jaisson P (2007). Individual Experience Alone Can Generate Lasting Division of Labor in Ants. *Current Biology* 17(15):1308–1312

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Reyes JL, Fernández Haeger J (1999). Sequential co-operative load transport in the seed-harvesting ant *Messor barbarus*. *Insectes Sociaux* 46:119–125

Riehl C, Frederickson ME (2016). Cheating and punishment in cooperative animal societies. *Philosophical Transactions of the Royal Society B* 371:20150090

Robinson EJH, Feinerman O, Franks NR (2009). Flexible task allocation and the organization of work in ants. *Philosophical Transactions of the Royal Society B* 276:4373–4380

Robinson EJH, Feinerman O, Franks NR (2012). Experience, corpulence and decision making in ant foraging. *Journal of Experimental Biology* 215:2653–2659.

Schmidt CA, Shattuck SO (2014). The higher classification of the ant subfamily Ponerinae (Hymenoptera: Formicidae), with a review of ponerine ecology and behaviour. *Zootaxa* 3817:1–242

Schöner TW (1971). Theory of feeding strategies. *Annual Review of Ecology and Systematics* 11:369–404

Schöner TW (1979). Generality of the Size-Distance Relation in Models of Optimal Feeding. *American Naturalist* 114(6):902–914

Sheppe W (1970). Invertebrate predation on termites of the African savanna. *Insectes Sociaux* 17:205–218

Simone-Finstrom MD, Spivak M (2012). Increased Resin Collection after Parasite Challenge: A Case of Self-Medication in Honey Bees? *PloS One* 7(3):e34601

Singer MS, Mace KC, Bernays EA (2009). Self-Medication as Adaptive Plasticity: Increased Ingestion of Plant Toxins by Parasitized Caterpillars. *PloS One* 4(3):e4796

Siva-Jothy MT, Moret Y, Rolff J (2005). Insect immunity: An evolutionary ecology perspective. *Advances in Insect Physiology* 32:1–48

Taylor K, Visvader K, Nowbahari E, Hollis KL (2013). Precision Rescue Behavior in North American Ants. *Evolutionary Psychology* 11:665–677

Theis FJ, Ugelvig LV, Marr C, Cremer S (2015). Opposing effects of allogrooming on disease transmission in ant societies. *Philosophical Transactions of the Royal Society B* 370:20140108

Tragust S (2016). External immune defence in ant societies (Hymenoptera: Formicidae): the role of antimicrobial venom and metapleural gland secretion. *Myrmecological News* 23:119–128

Tragust S, Mitteregger B, Barone V, Konrad M, Ugelvig LV, Cremer S (2013). Ants Disinfect Fungus-Exposed Brood by Oral Uptake and Spread of Their Poison. *Current Biology* 23(1):76–82

Traniello JFA (1989). Foraging Strategies of Ants. *Annual Review of Entomology* 34:191–210

Tranter C, Fernandez-Marin H, Hughes WOH (2015). Quality and quantity: transitions in antimicrobial gland use for parasite defense. *Ecology and Evolution* 5(24):857–868

Ugelvig LV, Cremer S (2007). Social prophylaxis: Group interaction promotes collective immunity in ant colonies. *Current Biology* 17(22):1967–1971

Vasconcelos M, Hollis K, Nowbahari E, Kacelnik A (2012). Pro-sociality without empathy. *Biology Letters* 8:910–912

Vaughan TA, Ryan JM, Czaplewski J (2010). Mammalogy 5th Edition. Jones & Bartlett Learning, Burlington, MA

Villet MH (1990). Division-of-Labor in the Matabele Ant Megaponera foetens (Fabr) (Hymenoptera-Formicidae). *Ethology Ecology & Evolution* 2:397–417

Wickham H (2009). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York, NY

Widgren BR, Jourak M (2011). Medical Emergency Triage and Treatment System (METTS): A New Protocol in Primary Triage and Secondary Priority Decision in Emergency Medicine. *The Journal of Emergency Medicine* 40(6):623–628

Wilson EO (1980). Caste and Division of Labor in Leaf-Cutter Ants (Hymenoptera, Formicidae, *Atta*). 1. The Overall Pattern in *Atta sexdens*. *Behavioral Ecology and Sociobiology* 7:143–156

Wilson EO (1984). The Relation between Caste Ratios and Division of Labor in the Ant Genus *Pheidole* (Hymenoptera, Formicidae). *Behavioral Ecology and Sociobiology* 16:89–98

Wilson EO (2014). A Window on Eternity: A Biologist's Walk Through Gorongosa National Park. Simon & Schuster. New York, NY

Witte V, Schliessmann D, Hashim R (2010). Attack or call for help? Rapid individual decisions in a group-hunting ant. *Behavioral Ecology* 21(5):1040–1047

Yusuf AA, Crewe RM, Pirk CWW (2013). An effective method for maintaining the African termite-raiding ant *Pachycondyla analis* in the laboratory. *African Entomology* 21:132–136

Yusuf AA, Gordon I, Crewe RM, Pirk CWW (2014). Prey choice and raiding behaviour of the Ponerine ant *Pachycondyla analis* (Hymenoptera: Formicidae). *Journal of Natural History* 48:345–358

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PUBLICATIONS

Published manuscripts

Frank ET, Schmitt T, Hovestadt T, Mitesser O, Stiegler J, Linsenmair KE (2017). Saving the injured: rescue behavior in the termite-hunting ant *Megaponera analis*, *Science Advances* 3:e1602187. doi: 10.1126/sciadv.1602187

Frank ET, Linsenmair KE (2017). Individual versus collective decision making: optimal foraging in the group-hunting termite specialist *Megaponera analis*, *Animal Behaviour* 130:27–35. doi: 10.1016/j.anbehav.2017.06.010

Frank ET, Linsenmair KE (2017). Flexible task allocation and raid organization in the termite-hunting ant *Megaponera analis*, *Insectes Sociaux*, **Online first**, doi: 10.1007/s00040-017-0579-2

Frank ET, Linsenmair KE (in press). Saving the injured: convergent evolution and mechanisms, *Communicative and Integrative Biology*, **in press**

Unpublished manuscripts

Frank ET, Wehrhahn M, Linsenmair KE (submitted). Saving the injured: wound treatment and selective help in the ant *Megaponera analis*. **Submitted.**

Hönle PO, **Frank ET**, Linsenmair KE (under review). Time optimized path-choice in the termite hunting ant *Megaponera analis*. **Under review.**

Frank ET, Fischer F, Linsenmair KE (in preparation). Emigration mechanisms and behaviour in the ponerine ant *Megaponera analis*. **In preparation.**

Frank ET, Schmitt T (in preparation). Temporal individual recognition in scouts of *Megaponera analis*. **In preparation.**

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12-13 Oct 2016; 11th International Symposium organised by the students of the Graduate School of Life Sciences (EUREKA), Würzburg, Germany
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6-10 Feb 2017; European Conference of Tropical Ecology (GTÖ), Brüssel, Belgium
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9 Mar 2017; Symposia of chemical ecology, Ulm, Germany
Contribution: Talk “Saving the injured: Rescue behaviour in the termite hunting ant *Megaponera analis*“

20-26 Aug 2017; Congress of the European Society for Evolutionary Biology (ESEB), Groningen, Netherlands
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Group Seminar

WS 2014/15 – WS2017/18; AG Schmitt, Prof. Dr. Thomas Schmitt, weekly, 1hr

Journal Club

WS 2014/15 – WS2017/18; AG Steffan-Dewenter, Prof. Dr. Ingolf Steffan-Dewenter, biweekly, 1hr

Jour Fixe

WS 2014/15 – WS2017/18; Zoology III PhD Meeting, Prof. Dr. Ingolf Steffan-Dewenter, monthly, 1hr

WS 2014/15 – WS2017/18; Zoology III Seminar, Prof. Dr. Ingolf Steffan-Dewenter, weekly, 1hr

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7- 11 Nov 2016; PhD course Social Evolution, Institute of Science and Technology, Austria

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Transferable Skills Courses

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4-5 Jul 2016; Oral presentation skills, Graduate School of Life Sciences, Würzburg, Germany

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2015 Charlotte Wenz	"Central place foraging theory applied to the termite hunting ant *Megaponera analis"*
2015 Philip Hönle	"Optimal trail choice and learning in the termite raiding ant *Megaponera analis*"
2016 Marten Wehrhahn	"Treatment and mortality causes of injured nestmates in the termite hunting ant *Megaponera analis*"
2017 Florens Fischer	"Emigration behaviour and mechanisms in *Megaponera analis*"
2017 Marius Pohl	"Population- and sociogenetic investigation in *Megaponera analis*"
2017 Vera Mayer	"Orientation mechanisms of *Megaponera analis* during raids"
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Figure	**Author Initials,** Responsibility decreasing from left to right				
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I also confirm my primary supervisor's acceptance.

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“Nothing in biology makes sense except in the light of evolution”
[Theodosius Dobzhansky: The American Biology Teacher, Vol. 35: 125–129, 1973]