Title: Wound treatment and selective help in a termite-hunting ant

Short Title: Wound treatment of injured ants

Authors: Erik. T. Frank,[1]* Marten Wehrhahn,[1] K. Eduard Linsenmair[1]

Published in Proceedings of the Royal Society B. DOI: 10.1098/rspb.2017.2457

Affiliations

[1]Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg. Am Hubland, D-97074 Würzburg.

*Correspondence to:

Erik Thomas Frank

E-mail: erik.frank@uni-wuerzburg.de

Tel: 0049/152 06060403

ORCID: 0000-0002-2066-3202

Address: University of Würzburg

Biocenter

Department of Animal Ecology and Tropical Biology (Zoology III)

Am Hubland

D-97074 Würzburg, Germany

Keywords: Rescue behaviour, social immunity, pro social behavior, myrmecology

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 (biting off 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 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 injured 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 of 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 included a differentiated treatment inside the nest.

INTRODUCTION

Open wounds are a major mortality risk in animals [1] 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 are generally 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) [2]. The benefit from helping injured ants in this scenario is small, since replacing them should be easier [3]. At the same time, if injuries were mainly fatal the benefit of a rescue behaviour focused on injured individuals would again be marginal [3]. 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 [3].

Megaponera analis is found in sub-Saharan Africa [4] and specialized on hunting termites solely from the subfamily Macrotermitinae [5-7]. 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 [5, 8-10]. 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 [11-13]. 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 [10, 13]. During the hunt some ants get injured by termite soldiers, which have strongly sclerotized heads and mandibles [14]. These ants often loose limbs or have termites clinging to them [3, 5, 15]. 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 trisulphide (DMTS) [3]. 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 [3]. If the injured ants were to return alone to the nest they would die in 32% of the cases during the return journey [3]. 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 [3]. These injuries occur regularly, with roughly a third of the minors participating in raids having lost a leg at one point in their life [3]. Saving the injured therefore significantly increases the fitness of the colony [3]. 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 [16]. Positive social interactions – e.g. preventing the spread of an infection through adaptive behaviour – may more than compensate the system beyond the single individual immune competence: social immunity [16, 17]. This can range from purely prophylactic behaviours like removing corpses and waste from the nest [18], using antimicrobial substances as nest material [19] or actively grooming nestmates to keep their cuticles free from parasites [20]. One of the main chemical defences against infections in ants are the secretions of the metapleural and venom gland [21, 22]. These glands excrete antimicrobial substances, which during allogrooming by nestmates get spread over the cuticle and thus inhibit infections [21-23]. While individuals that suffer from parasites receive more (or depending on infectiousness less) attention from nestmates [24], 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?

METHODS

Experimental design. The study was conducted in a humid savannah woodland located in the Comoé National Park [25], 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 [10]). *Megaponera analis* is found throughout sub-Saharan Africa from 25°S to 12°N [4]. We observed 208 raids of 16 different colonies of *M. analis* on which the predominantly hunted termite genus was *Pseudocanthotermes* [10]. Colony size for 14 excavated colonies was between 900-2300 ants, a result comparable to previous studies in other regions [11, 26]. *Megaponera analis* is known to show monophasic allometry within its worker sizes (i.e. an elementary form of polymorphism: most body parts are isometric but a few are allometric) [11, 12]. We thus divided the workers into majors (head width > than 2.40 mm), minors (head width < 1.99 mm) and intermediates (head width 2.40 - 1.99 mm) for Fig. S1, as proposed by Villet [11]. All field studies were conducted in accordance with local legislation and permission by the Office Ivoirien des Parcs et Réserves (OIPR).

FIELD EXPERIMENTS

Selective help dependent on injury severity. To test if the rescue behavior was dependent on injury severity (loss of two or five legs) we presented returning raids with differently manipulated injured individuals. The experiments were each repeated 20 times with at least five different colonies per experiment, with the same protocol as in Frank et al. [3]. 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. The ant for a trial was collected during the outward journey of the raid and manipulated during the hunting phase, frozen dummies were also collected from a raiding party (of the same colony) at least 24 hours before the experiment. The pheromone was applied on a glass surface over which we pulled the thorax of the dummy three times. Heavily injured ants had 5 legs randomly removed with scissors at the femur. To incapacitate the legs without removal they were crushed with a pair of forceps. All behavioral reactions by the nestmates were recorded until the whole column had passed the study subject or it was carried back/away. The behavioral 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 away from the column and not in the direction of the nest. For statistical analysis we only identified behavior 4 (carried back) as a successful rescue behavior. Data for lightly injured ants (2 legs experimentally removed) and dummy were taken from Frank et al. [3]. 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 individual was also quantified.

Visual reinforcement of injury. We wanted to test if injured ants behaved differently dependent on nestmate proximity/presence. 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 center of the returning raid column or on the return pheromone trail one minute after the raid column had passed. The same experiment was conducted with uninjured ants as a control. Each raid was only used for one experiment (n=20 per experiment for n=80 raids). We measured the distance an ant travelled in 60 s to calculate running speed (cm/s). 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, which was done for a total of 82 raids.

To see what type of injury was picked up at the hunting ground or during 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 eight raids in three different colonies.

LABORATORY EXPERIMENTS

Laboratory colonies. Six colonies were excavated and placed in artificial nests in the field stations laboratory (colony size 1293±543 ants), including queen and brood. Nests (30x20x10 cm) were made of PVC and connected to a 1x1m feeding arena. The ground and nest was covered with soil from the surrounding area (up to a height of 2cm). 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. Since the laboratory was in the national park, humidity, temperature and day cycle (light schedule) was the same as in nature (open windows), experiments were only started during the day/activity period. For further details on lab keeping see Yusuf et al. [26].

To quantify the percentage and severity of injured ants in a colony all individuals were carefully examined for any lost extremities (directly after excavation of the colony) and then returned to the nest (in total 7240 ants were analysed in six colonies).

Treatment of wounds by nestmates. We wanted to quantify how injured ants were treated inside the nest by nestmates. 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, collected at foraging sites in the vicinity of the station) 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) wound grooming: a nestmate cleans the open wound with its mouthparts; (3) allogrooming: the subject is 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.

Survival of injured ants. To quantify the value of the treatment isolation trials were conducted. For these 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 surface soil from the same location near the research station up to a height of 1 cm. To create nest like humidity conditions the soil was moistened with 1 ml of sterilized water (boiled for ten minutes) and covered with aluminium foil. The experiments were conducted at 24°C. 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 injured ant was then placed in the container and checked once per hour 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 [27] with the user interface RStudio v0.98.501 and the R package ggplot2 v2.1.0 [28]. We tested for deviations from the normal distribution with the Shapiro Wilks test ($p>0.05$). A Bartlett test was used to verify homoscedasticity ($p>0.05$), this was not the case for all our data. For the nest treatment experiments a generalized linear mixed-effects model (GLMM) was used for the relationship between the quantity of a shown behaviour (wound grooming, antennation, allogrooming, pulling, biting) and time. Fixed effects were the time categories (in 30min intervals) and in the case of antennation and allogrooming also as interaction with the treatment type (lightly injured, heavily injured, termite bite, healthy). As random effects we included the colony and trial (nested in colony). A linear mixed effect model (LMM) was used for Gaussian distributed data (not count) with colony as a random factor. P-values were obtained by likelihood ratio tests of the full model with the effect in question against an intercept only model. To analyse the ethogram data a Fisher's exact test with Holm-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 mixed effect cox proportional hazards regression model with colony as a random factor and an overall likelihood ratio test against an intercept only model. For post-hoc analyses of the models least-square means were compared using the R package lsmeans with a Holm-Bonferroni correction. 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

Selective help dependent on injury severity. In the six excavated 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. Minors and intermediates made up the majority of injured ants (Fig. S1; LMM: X^2_2=49.6; p<0.001; Random effects: Colony: Variance=0, Std. Dev.=0; Residual: Variance=0.24, Std. Dev.=0.49; ls means: once vs twice: Z=7.1, p<0.001; once vs thrice: Z=8.0, p<0.001; twice vs thrice: Z=0.93, p=0.35).

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 back 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. 1a). Interestingly nestmates investigated heavily injured ants significantly longer than lightly injured ants (Fig. 1b). To rule out potential leg counting as the selective mechanism 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. 1a). Applying the synthesized help pheromone DMDS and DMTS on a heavily injured ant significantly increased the number of pick up attempts and carried ants (Video S1, Fig. 1a and Table S1). The video material of heavily injured ants did not reveal cooperative behaviour by them towards the helper (Video S1). The heavily 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. 1b and Table S2).

Visual reinforcement of injury. Even though all injuries were 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 next to 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. 2 and Table S3). 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 at a faster pace (Video S2).

Treatment of wounds by nestmates. Handicapped ants were antennated 110% more often than healthy control ants during the first hour after injury (Fig. S2a and Table S4). Injured ants were frequently groomed directly at the injury within the first hour (Fig. 3a and Table S5). The remaining part of the cut limb was 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. S3ab and Video S3). Ants with clinging termites had nestmates pulling on the termite, with the handicapped ant pulling in the opposite direction (Fig. 3b and Table S6). Nestmates often bit the termite, specifically on the area of the pronotum. This behaviour led to the removal of the termite body, with the head remaining in place (Fig. S3c). In three cases the termite was removed completely within 60 min, in two further cases within 24 hours and in five trials the termite was not removed (n=10). In one case the termite head remained clinging on the ant even two weeks later (termite body was removed).

The majority of allogrooming by nestmates was concentrated on the acrylic colour marking on the ant and the number of these interactions remained relatively constant throughout the 3 hours of observation, with a small peak in the first 30 min (Fig. S2b and Table S7). Nestmates were observed carrying heavily injured ants out of the nest within the first 30 min of the trial and since the heavily injured ants did not return to 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 (Fig. S4; 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. S4 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 of injured ants. To test for possible benefits of the treatment on lightly injured ants we isolated minors that had two extremities cut off. On unsterile soil the injured ants had a mortality of 80% within the first 24 hours (n=20; Fig. 4 and Table S8), while the mortality was only 10% when the injured ants had received a one or twelve hour treatment beforehand by their nestmates inside the nest (n=20; Fig. 4). 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. 4). Furthermore, a freshly cut wound appeared to be completely sealed/clotted within ten minutes, without interaction by nestmates in a controlled environment (Fig. S5).

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 [3], 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, likely through a passive decision-making process. Lastly we show that injured ants change their behaviour according to the proximity of nestmates.

Selective help dependent on injury. Ants that lost extremities made up 5% of the colony, this is in stark contrast to the 21% they make up in the raiding party [3]. This discrepancy probably has multiple causes. The age polyethism in *M. analis* leads to younger ants being focused mostly on nest tasks [11], 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 [29, 30], this could also hold true for *Megaponera 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. 1). 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 (Video S1). Lightly injured ants immediately assumed a pupae-like position when antennated by a nestmate, 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 (Video S1), 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. 1b), 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. 1a), 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 most likely due to the uncooperativeness by the injured ant itself.

This is further supported by the lack of treatment and absence of heavily injured ants inside the nest and heavily injured ants leaving the nest or being carried out within the first hour. This behaviour is very similar to moribund ants leaving the nest when parasitized or close to death [31, 32] and has also been previously observed to occur in *M. analis*, with injured ants leaving the nest [15], 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* [33] or *Myrmica rubra* [34]. 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 [34]. This could also explain our observations in heavily injured ants (Video S1). Another mechanism in honeybees and ant brood are chemical sickness cues emitted by the infected individual, thus leading to antagonistic behaviour by nestmates and removal from the colony [35, 36], this was not tested for in our study.

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. These results are in line with prior studies concerning rescue behaviour [37, 38] and support the hypothesis for the evolution of pro-social behaviour without the necessity of empathy or cognition [39, 40].

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 is 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. If no nestmates are present a fast return speed by the injured individual should reduce its risk of being predated. 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. 2). 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 would 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 [41], 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)[3]. The fact that heavily injured ants do not seem to call for help (Fig. 1a) and are not found inside the nest (Fig. S1) further underscores the argument against cheating.

Treatment of wounds by nestmates. 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 [1]. Injuries occur at termite foraging sites [3] 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 (Fig. 4). The treatment by nestmates was clearly focused on the wound and led to intense grooming directly into the open wound (Fig. S3 and Video S3), 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 has been observed in various species, from a wide range of taxa [42]. In primates self-medication has been observed, by including medicinal plants in their diet when sick [43] but also includes mutual medication in capuchin monkeys as topically applied anti-parasite substances [44]. In social insects social immunity and cooperation play a crucial role when confronted with parasites [16]. Wood ants (*Formica paralugubris*) use antimicrobial resin in their nests as prophylaxis [45] and honey bees (*Apis mellifera*) even increase resin collection pro-actively when parasitized [46]. There are many more examples of colony responses and organization to parasite infections on a colony level [16, 47], our observations are more focused on the level of the individual. It has been previously shown that ants disinfect fungus-exposed brood through allogrooming [48] and that grooming overall leads to parasite reduction on treated individuals [49, 50]. 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 in the individual 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. In addition, the treatment might include antimicrobial substances being applied on the wound. Ants have been shown to wound their infected brood and then spray antimicrobials into those wounds to kill infections (and the brood in the process) [35], although in our study the behaviour is protective rather than sacrificial. The fact that wound clotting also seems to occur remarkably fast (within ten minutes, Fig. S5) 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 highly effective 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 and how does wound clotting affect treatment? We hope that further research will help answer these questions.

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).

Data accessibility. Data used in this study is available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.tq2qh

Acknowledgements. We thank V. Frank, B. Fiala and the anonymous referees for critical discussions and reading the manuscript. We thank H. Feldhaar for her advice and J. Rohde for his help on the isolation trials and statistics. We thank the Comoé National Park Research Station for the use of their facilities for the field and laboratory research and the park management of Office Ivoirien des Parcs et Réserves for facilitating field research in the park.

Author contributions. E.T.F. and K.E.L. designed the study. E.T.F. collected, analysed the field data and wrote the paper. M.W. conducted part of the laboratory experiments. K.E.L. supervised the study. All authors discussed the results and commented on the manuscript.

FIGURE LEGENDS

Fig. 1 Injury severity dependent help. (**A**) Rescue behaviour in nestmates of *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 (gray). 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 S1 for detailed statistical results); n=20. Data for light and dummy trials from Frank et al. 2017 [3]. (**B**) Investigation time by nestmates on injured individual. Dead: frozen dead ant; Helped: Time of investigation for ants that were helped. LMM followed by a least square means analysis; n=20. See table S2 for detailed statistical results.

Fig. 2 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). LMM followed by a least square means analysis; n=20. See also table S3 for detailed statistical results.

Fig. 3. Treatment of handicapped and injured ants inside the nest. (**A**) Number of times wound grooming by nestmates on injured ants (two cut off limbs) was observed; n=10. (**B**) 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. GLMM followed by a least square means analysis (see also table S5 and S6 for detailed statistical results).

Fig. 4. Survival probability of injured ants. Kaplan-Meier cumulative survival rates of workers in isolation that received different treatments. 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; 1h-treatment: ant with two removed limbs kept in the nest for 1 hour before being isolated on unsterile earth. N=20 for all experiments. ***:$p<0.001$. Statistical significance tested with a Mixed effects Cox proportional hazards regression model (Table S8) followed by a post hoc least square means analysis.

REFERENCES

1. Siva-Jothy MT, Moret Y, Rolff J. 2005 Insect immunity: An evolutionary ecology perspective. *Adv Insect Physiol* **32**, 1-48. (doi:10.1016/S0065-2806(05)32001-7).

2. Hölldobler B, Wilson EO. 1990 *The Ants*, Belknap Press of Harvard University Press.

3. Frank ET, Schmitt T, Hovestadt T, Mitesser T, Stiegler J, Linsenmair K.E. 2017 Saving the injured: Rescue behavior in the termite hunting ant *Megaponera analis*. *Sci Adv* **3**, e1602187. (doi:10.1126/sciadv.1602187).

4. Schmidt CA, Shattuck SO. 2014 The Higher Classification of the Ant Subfamily Ponerinae (Hymenoptera: Formicidae), with a Review of Ponerine Ecology and Behavior. *Zootaxa* **3817**(1), 1-242.

5. Yusuf AA, Gordon I, Crewe RM, Pirk CWW. 2014 Prey choice and raiding behaviour of the Ponerine ant *Pachycondyla analis* (Hymenoptera: Formicidae). *J Nat Hist* **48**(5-6), 345-358. (doi:10.1080/00222933.2013.791931).

6. Longhurst C, Johnson RA, Wood TG. 1978 Termite Predation by *Megaponera-foetens* (Fabr) (Hymenoptera - Formicidae) on termites in Nigerian southern Guinea savanna. *Oecologia* **32**(1), 101-107. (doi:10.1007/Bf00344694).

7. Levieux J. 1966 Note préliminaire sur les colonnes de chasse de *Megaponera fœtens* F. (Hyménoptère Formicidæ). *Ins Soc* **13**(2), 117-126. (doi:10.1007/bf02223567).

8. Bayliss J, Fielding A. 2002 Termitophagous foraging by *Pachycondyla analis* (Formicidae, Ponerinae) in a Tanzanian coastal dry forest. *Sociobiology* **39**(1), 103-122.

9. Hölldobler B, Braun U, Gronenberg W, Kirchner WH, Peeters C. 1994 Trail communication in the ant *Megaponera foetens* (Fabr.) (Formicidae, Ponerinae). *J Insect Physiol* **40**(7), 585-593. (doi:10.1016/0022-1910(94)90145-7).

10. Frank ET, Linsenmair KE. 2017 Individual versus collective decision making: optimal foraging in the group hunting termite specialist *Megaponera analis*. *Anim Behav* **130**, 27–35.

11. Villet MH. 1990 Division-of-Labor in the Matabele Ant *Megaponera foetens* (Fabr) (Hymenoptera-Formicidae). *Ethol Ecol Evol* **2**(4), 397-417.

12. Crewe RM, Peeters CP, Villet M. 1984 Frequency-distribution of worker sizes in *Megaponera foetens* (Fabricius). *S Afr J Zool* **19**(3), 247-248.

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

14. Prestwich GD. 1984 Defense-mechanisms of termites. *Annu Rev Entomol* **29**, 201-232.

15. Burgeon L. 1929 Une organisation sanitaire chez les fourmis *Megaponera*. *Rev Zool Bot Afr* **16**, 94-95.

16. Cremer S, Armitage SAO, Schmid-Hempel P. 2007 Social immunity. *Curr Biol* **17**(16), 693-702. (doi:10.1016/j.cub.2007.06.008).

17. Meunier J. 2015 Social immunity and the evolution of group living in insects. *Phil Trans R Soc B* **370**(1669), 20140102. (doi:10.1098/rstb.2014.0102).

18. Howard DF, Tschinkel WR. 1976 Aspects of Necrophoric Behavior in Red Imported Fire Ant, *Solenopsis invicta*. *Behaviour* **56**, 157-180. (doi:10.1163/156853976x00334).

19. Chouvenc T, Efstathion CA, Elliott ML, Su NY. 2013 Extended disease resistance emerging from the faecal nest of a subterranean termite. *Proc Royal Soc B* **280**(1770), 20131885. (doi:10.1098/rspb.2013.1885).

20. Oi DH, Pereira RM. 1993 Ant Behavior and Microbial Pathogens (Hymenoptera, Formicidae). *Fla Entomol* **76**(1), 63-74. (doi:10.2307/3496014).

21. Fernandez-Marin H, Zimmerman JK, Rehner SA, Wcislo WT. 2006 Active use of the metapleural glands by ants in controlling fungal infection. *Proc Royal Soc B* **273**(1594), 1689-1695. (doi:10.1098/rspb.2006.3492).

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

23. Tranter C, Fernandez-Marin H, Hughes WOH. 2015 Quality and quantity: transitions in antimicrobial gland use for parasite defense. *Ecol Evol* **5**(24), 857-868. (doi:10.1002/ece3.1827).

24. Theis FJ, Ugelvig LV, Marr C, Cremer S. 2015 Opposing effects of allogrooming on disease transmission in ant societies. *Philos Trans R Soc B* **370**(1669), 20140108. (doi:10.1098/rstb.2014.0108).

25. Konaté S, Kampmann D. 2010 *Biodiversity atlas of West Africa, Volume 3: Côte d'Ivoire*. Abidjan & Frankfurt am Main.

26. Yusuf AA, Crewe RM, Pirk CWW. 2013 An effective method for maintaining the African termite-raiding ant *Pachycondyla analis* in the laboratory. *Afr Entomol* **21**(1), 132-136.

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

28. Wickham H. 2009 *ggplot2: Elegant Graphics for Data Analysis*, Springer-Verlag New York.

29. Moron D, Lenda M, Skorka P, Woyciechowski M. 2012 Short-Lived Ants Take Greater Risks during Food Collection. *Am Nat* **180**(6), 744-750. (doi:10.1086/668009).

30. Moron D, Witek M, Woyciechowski M. 2008 Division of labour among workers with different life expectancy in the ant *Myrmica scabrinodis*. *Anim Behav* **75**, 345-350. (doi:10.1016/j.anbehav.2007.06.005).

31. Heinze J, Walter B. 2010 Moribund Ants Leave Their Nests to Die in Social Isolation. *Curr Biol* **20**(3), 249-252. (doi:10.1016/j.cub.2009.12.031).

32. Chapuisat M. 2010 Social Evolution: Sick Ants Face Death Alone. *Curr Biol* **20**(3), 104-105. (doi:10.1016/j.cub.2009.12.037).

33. Miler K. 2016 Moribund Ants Do Not Call for Help. *PloS one* **11**(3), e0151925. (doi:10.1371/journal.pone.0151925).

34. Leclerc JB, Detrain C. 2017 Loss of attraction for social cues leads to fungal-infected *Myrmica rubra* ants withdrawing from the nest. *Anim Behav* **129**, 133-141. (doi:10.1016/j.anbehav.2017.05.002).

35. Pull CD, Ugelvig LV, Wiesenhofer F, Tragust S, Schmitt T, Brown MJ, Cremer S. (In press) Destructive disinfection of infected brood prevents systemic disease spread in ant colonies. *eLife*

36. Richard FJ, Aubert A, Grozinger CM. 2008 Modulation of social interactions by immune stimulation in honey bee, Apis mellifera, workers. *BMC Biol* **6**, 50. (doi:10.1186/1741-7007-6-50)

37. 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

38. Nowbahari E, Hollis KL. 2010 Rescue behavior: Distinguishing between rescue, cooperation, and other forms of altruistic behavior. *Commun Integr Biol*, 3, 77–79.

39. Vasconcelos M, Hollis K, Nowbahari E, Kacelnik A. 2012 Pro-sociality without empathy. *Biol Lett* **8**(6), 910-912. (doi: 10.1098/rsbl.2012.0554).

40. Frank ET, Linsenmair KE. 2017 Saving the injured: Evolution and mechanisms. *Commun Integr Biol*, e:1356516. (doi:10.1080/19420889.2017.1356516)

41. Riehl C, Frederickson ME. 2016 Cheating and punishment in cooperative animal societies. *Philos Trans R Soc B* **371**(1687), 20150090. (doi:10.1098/rstb.2015.0090).

42. de Roode JC, Lefevre T, Hunter MD. 2013 Self-Medication in Animals. *Science* **340**(6129), 150-151. (doi:10.1126/science.1235824).

43. Huffman MA. 2003 Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. *P Nutr Soc* **62**(2), 371-381. (doi:10.1079/Pns2003257).

44. Bowler M, Messer EJE, Claidière N, Whiten A. 2015 Mutual medication in capuchin monkeys – Social anointing improves coverage of topically applied anti-parasite medicines. *Sci Rep* **5**, 15030. (doi:10.1038/srep15030)

45. Castella G, Chapuisat M, Christe P. 2008 Prophylaxis with resin in wood ants. *Anim Behav* **75**, 1591-1596. (doi:10.1016/j.anbehav.2007.10.014).

46. 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. (doi:10.1371/journal.pone.0034601).

47. Ugelvig LV, Cremer S. 2007 Social prophylaxis: Group interaction promotes collective immunity in ant colonies. *Curr Biol* **17**(22), 1967-1971. (doi:10.1016/j.cub.2007.10.029).

48. 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. *Curr Biol* **23**(1), 76-82. (doi:10.1016/j.cub.2012.11.034).

49. Pritchard DJ. 2016 Grooming by honey bees as a component of varroa resistant behavior. *J Apicult Res* **55**(1), 38-48. (doi:10.1080/00218839.2016.1196016).

50. Hughes WOH., Eilenberg J, Boomsma JJ. 2002 Trade-offs in group living: transmission and disease resistance in leaf-cutting ants. *Proc Royal Soc B* **269**(1502), 1811-1819. (doi:10.1098/rspb.2002.2113).