BEHAVIORAL ECOLOGY

Saving the injured: Rescue behavior in the termite-hunting ant *Megaponera analis*

Erik Thomas Frank,* Thomas Schmitt, Thomas Hovestadt, Oliver Mitesser, Jonas Stiegler, Karl Eduard Linsenmair

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 (in this study, mostly colonies of *Pseudocanthotermes* sp.) at their foraging sites. The evolutionary arms race between termites and ants led to various defensive mechanisms in termites (for example, a caste specialized in fighting predators). Because *M. analis* incurs high injury/mortality risks when preying on termites, some risk-mitigating adaptations seem likely to have evolved. We show that a unique rescue behavior 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 that are forced experimentally to return without help, die in 32% of the cases. Behavioral experiments show that two compounds, dimethyl disulfide and dimethyl trisulfide, present in the mandibular gland reservoirs, trigger the rescue behavior. A model accounting for this rescue behavior identifies the drivers favoring its evolution and estimates that rescuing enables maintenance of a 28.7% larger colony size. Our results are the first to explore experimentally the adaptive value of this form of rescue behavior focused on injured nestmates in social insects and help us to identify evolutionary drivers responsible for this type of behavior to evolve in animals.

INTRODUCTION

Helping behavior has been observed throughout the animal kingdom, from social insects to primates (1). Rescue behavior observed in ants can arise in 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 (2–5). All hitherto observed types of rescue behavior in social insects were always directed toward individuals under an imminent threat (1, 6, 7), that is, suffocation or being eaten. *Megaponera analis* is a strictly termitophagous ponerine ant species, found in sub-Saharan Africa from 25°S to 12°N (8) that specializes in raiding termites of the subfamily Macrotermitinae at their foraging sites (9–13). 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 to 500 nestmates and lead them to the termites in a column-like march formation, which can be up to 50 m away from the nest (11, 13, 14). During the raid, division of labor occurs (15): larger ants (majors) break open the protective soil cover created by the termites, whereas the smaller ants (minors) rush into these openings to kill and pull out the termite-hunting ant *Megaponera analis* is a strictly termitophagous ponerine ant species, found in sub-Saharan Africa from 25°S to 12°N (8) that specializes in raiding termites of the subfamily Macrotermitinae at their foraging sites (9–13). 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 to 500 nestmates and lead them to the termites in a column-like march formation, which can be up to 50 m away from the nest (11, 13, 14). During the raid, division of labor occurs (15): larger ants (majors) break open the protective soil cover created by the termites, whereas the smaller ants (minors) rush into these openings to kill and pull out the termite clinging on to ant extremities in the ant nest, and the rescue behavior toward ants that carry long-term injuries in the form of lost extremities. This specialized rescue behavior is unanticipated in insects, where the value of individuals is generally underestimated, and could provide further proof that empathy is not necessary for helping behavior to emerge in animals (21).

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 (movie S1 and fig. S1A). On an average raid, a median of 3 ± 2.9 ants (of 416 ± 153 ants) were carried back (*n* = 53 raids with 154 carried ants), for a total of 9 to 15 rescued ants per day (3 to 5 raids per day). Only in 11% (6 of 53) of the raids were no ants carried back to the nest, 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 the “Quantification of model” section in Materials and Methods), the rescued ants make up a large proportion of the daily turnover in the colony.

Value of rescue behavior 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 to their bodies, and (iii) ants that appear to carry no obvious injury (fig. S1B). Most carried ants had a termite clinging on an extremity (Fig. 1A and table S1). This handicap reduces the speed of the ant the most (4.5% of the mean speed of a healthy individual; Fig. 1B and table S1) and, if removed successfully, has no long-term consequences. 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 of 60) of them died (Fig. 2A), in contrast to 10% of healthy individuals (*n* = 2 of 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

---

*Corresponding author. Email: erik.frank@uni-wuerzburg.de*

Department of Animal Ecology and Tropical Biology, Biocentre, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany.
Focus of rescue behavior

To show that this behavior is 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. Whereas 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. 5A, table S4, and movie S1). Artificially injured individuals from other colonies were always attacked and removed from the column (Fig. 5A and table S4). Rescue behavior 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. 5B and table S4).

Gland and pheromone triggering rescue behavior

When looking for the signal triggering this rescue behavior, we first ruled out stridulation (22), a mechanism known to trigger helping behavior in other ants (I, 7). We observed artificially injured ants, on which stridulation was inhibited, to still be rescued (P < 0.001; n = 20; fig. S2, A and B, and table S4). After extensive behavioral experiments on dummies (frozen minors), we were able to identify the mandibular gland as the most likely candidate, while ruling out hindgut content and the Dufour’s and poison gland reservoirs as triggers of the behavior. To further test this hypothesis, we applied the mandibular gland contents onto healthy individuals; we found that healthy ants covered with mandibular gland material were then carried back by their nestmates (Fig. 5C and table S4). A gas chromatography–mass spectrometry (GC-MS) analysis identified dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) as the main chemical components of the gland, confirming a previous analysis of the gland contents and concentrations (14 ng of DMDS and 5 ng of DMTS per gland) (10). Although 9 ng of DMDS alone was not enough to trigger the rescue behavior on a dummy, 9 ng of DMTS by itself sufficed. A more pronounced response was achieved with an equal mixture of the two components (9 ng of DMDS and DMTS each) (Fig. 5D and table S4).
Value of rescue behavior for the colony

The rescue behavior in *M. analis* reduces the foraging costs through a reduced mortality risk. We provide a simple analytical model (additional information in “Rescue behavior model” and “Quantification of model” sections in Materials and Methods) that identifies critical factors promoting the evolution of this rescue behavior and why it may have evolved so rarely.

We consider that this behavior 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 this rescue behavior: (i) the product of the absolute rate at which ants are severely injured (or killed) in conflict with termites ($e_{1f}$) and the fraction $f$ ($0 \leq f \leq 1$) of these ants that could profit from the rescue behavior; (ii) the baseline mortality $\mu_0$ of ants—helping is more profitable if $\mu_0$ is small compared to $e_{1f}$; and (iii) the future added mortality rate ($\mu_J$) of individuals that were injured and rescued.

The foraging behavior of *M. analis* seems to offer ideal conditions for rescue behavior to arise. Injury rates in combat ($e_{1f} = 0.17\%$ per day; for detailed calculations, “Quantification of model” section in Materials and Methods) seem to be large compared to the general mortality rate ($\mu_0 = 0.76\%$ per day), but injuries are rarely fatal (six observations of fatal injuries in 53 raids). Further, ants that recently lost a leg or had termites clinging on their 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 signaling a vulnerable state. These effects result in a high mortality risk of 32% for injured individuals if not helped (Fig. 2A). Carried injured individuals therefore benefit greatly from the rescue behavior, by reducing that risk to close to 0% (injured ants that were marked and rescued were observed again in subsequent raids in 38 of 40 cases and were never observed to die during the rescue process). Injured ants that are carried back recover from injuries in a short time, that is, parameter $\mu_J$ is close to $e_{1f}$, 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. 4) substantiates the great value of helping injured nestmates, a conservative estimate because nonpermanent injuries are not included in this estimate. The value of rescue behavior is reflected in the sustainable colony size calculated by our model, which predicts a 28.7% larger colony size compared to colonies without this behavior.

DISCUSSION

This study shows the adaptive value of rescue behavior in a social predator specialized on a highly defensive prey—a behavior 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 behavior is induced by pheromones, we support the hypothesis that the convergent evolution
of rescue behavior in different taxa has led to distinctive triggering mechanisms, such as chemical communication in insects or empathy in humans and possibly other mammals ([23, 24]; but see the study of Vasconcelos et al. (21) for other interpretations).

Rescuing injured individuals

Intermediates and minors carry injuries considerably more often than majors (Fig. 4). The division of labor at the hunting ground could explain this discrepancy. Whereas 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 (16). Minors and intermediates are therefore far more exposed to injury risks.

A considerable number of carried ants did not seem to be injured (Fig. 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 leftover termites or injured individuals are less selective in what to carry. If they still encounter a minor, which might have lagged behind because it is 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 toward the termites seems to suggest that the behavior is context-specific (Fig. 5B). It seems unlikely for the ants to ever encounter this situation naturally. Furthermore, the rescue behavior 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 it carried back to the nest afterward. The different response necessitated 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 behavior is triggered through the chemical compounds DMDS and DMTS harbored in the mandibular gland reservoir; thus, this suite of compounds is a pheromone that seems to be specifically released when the individual is injured, in order to induce rescue behavior. The only other known species harboring this pheromone is the solitary hunting ponerine ant Paltothyreus tarsatus, in which the pheromone triggers digging behavior, most likely to rescue trapped nestmates (25). This species is in the same genus group as Megaponera (8), but being a solitary forager, P. tarsatus probably has not evolved the same kind of cooperative foraging-injury rescue behavior as in M. analis.

Cooperative self-defense has also been observed in M. analis, as a behavior in which nestmates scanned each other’s legs and antennae and removed Dorylus sp. (driver ants) clinging to their extremities during encounters (26). The removal of these Dorylus ants seems to follow a mechanism similar to the removal of termite soldiers within the nest.

Evolution of helping the injured

We were able to assess the value of rescue behavior for injured individuals. Because assistance to individual ants is the main benefit of the rescue behavior, understanding the evolutionary benefit of this behavior for the colony as a whole is paramount. Ants that had lost an extremity do not immediately switch to a four- or five-legged locomotion mechanism but keep tripping over their phantom limbs. Ants that had termites clinging on them were even more severely handicapped in their movement (Fig. 1B). These ants were therefore unable to keep up with the returning column, fell behind, and thus became isolated from their nestmates. This retarded movement, on top of reduced dexterity, increased predation risk considerably (Fig. 2, A to E). Once termites clinging on 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,
species must be able to inflict a high amount of nonlethal injuries from which the ants can recover. With their large soldiers, termites fulfill this criterion as injury-inducing prey. Group foraging ant species that focus on leaf cutting, nectar, seeds, or scavenging are less likely to develop this rescue behavior of termite-raiding ants. Fourth, 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; because minors sustain the most injuries (Fig. 4), the additional task for the majors to carry them back seems minimal from an energetic point of view (fig. S1A). Moreover, because on an average raid only 30% of the ants carry back prey (II), a large part of the workforce is available to help the injured individuals without decreasing the profits of the raid. Because the cost of helping an injured ant is therefore likely to be marginal in *M. analis*, it is thus ignored in our model. Last, 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 life span 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, such as army ants, seem likely to derive less

Fig. 5. Behavioral responses of helper ants toward 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 (gray). Negative values show behavior 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 toward different injury states. (B) Response at different points of the raid (Way out: on the way toward the termites; Hunting: at the hunting ground; Return: on the return journey after the fight). (C) Response toward 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 toward dummies treated with different synthetic compounds (DMDS/DMTS: 50:50 mixture of DMDS and DMTS; Hexane: pure hexane as control). See also table S4 for detailed statistical results. *P < 0.05; **P < 0.01; ***P < 0.001. n.s., not significant.

to recover from their injury in the safe environment of the nest, allowing them to get accustomed to a four- or five-legged locomotion. Thus, they reached running speeds similar to those of uninjured ants 24 hours later (Fig. 3). Because 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 behavior, 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 behavior can only evolve in group-hunting species, where an injured ant can be detected by its nestmates, it excludes all solitary hunting behavior can only evolve in group-hunting species, where an injured individual without decreasing the profits of the raid. Because the ants wait after the outward and return journeys are conducted as a discrete column rounded by nestmates warding off potential predators. In *M. analis*, the outward and return journeys are conducted as a discrete 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 (11, 15) exemplifies the importance of returning as a group. Third, the prey
benefit from saving one injured ant, although this hypothesis 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 behavior evolved as part of an evolution-
ary arms race against termites, as a means of minimizing losses during
raids and therefore foraging costs.

Rescue behavior has been previously observed in ants (1) but in
very different contexts. Excavating trapped nestsmates after a cave-in and
rescuing an ant that fell in an antlion trap are both situations in which
the individuals are confronted with an imminent danger, that is, suff-
focation or being eaten (1, 3, 5). 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 immediate danger toward 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 study demonstrates that complex rescue behavior 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 behavior in
animals: Injury and predation rates can be manipulated, rescue be-
behavior can easily be prevented, and critical variables and parameters
can be measured. The Pan-African distribution of *M. analis* should
also allow us to study the degree of fine-tuned adaptations to differing
external selection pressures prevailing in different ecosystems and en-
able identification of the most important potential driving factors for
evolution of this behavior. Our model also helps us to identify other
candidate species in which this behavior might be found. Other po-
erine genera, such as *Leptogenys*, also focus on hunting termites, with
some of them hunting in groups (27); examining their raiding behav-
ior in more detail could be promising. Slave-making ants could poten-
tially also fulfill the criteria, if their prey can inflict a significant amount
of nonlethal injuries.

**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) (28). Observa-
tions throughout several days in April 2013 established that raiding
activity was highest in the morning and afternoon hours between 0600
to 1100 and 1500 to 1900 local time, which corresponds to previous
observations (11, 13, 14). Night raiding was also observed but was
not included in this study. Experiments and observations were carried
out in the field from 0700 to 1100 and 1500 to 1800 from April to
September 2013, August to October 2014, and January to March and
July to November 2015. *M. analis* is found throughout sub-Saharan
Africa from 25°S to 12°N (8). We observed *M. analis* in a total of 52
different colonies for a total of 420 raids, in which the predominantly
hunted termite species was *Pseudacanthotermes* sp. Living nests of
*Macrotermes bellicosus*, which in other areas were often favored 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 of 10 excavated colonies ranged between 900 and
2300 ants, a result comparable to previous studies in other regions
(16, 29). Although *M. analis* is known to show monophasic allometry
within its worker sizes (16, 30) for statistical analysis and illustration, the
workers were divided into majors (head width, >2.40 mm), minors (head
width, <1.99 mm), and intermediates (head width, 2.40 to 1.99 mm), as
proposed by Villet (16).

**Quantification of carried ants**

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 ex-
periments 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 in the carried ants (that is, with the following
categories: lost limb, carried unharmed, and termite clinging), we re-
trieved the ants with forceps and investigated them. Injury inspections
were conducted in 20 raiding columns of 20 different colonies for a total
of 154 carried ants (Fig. 1A and fig. S1B).

To quantify the number of long-term injured ants participating in
raids, we collected ants of all castes from 20 raids, each from a differ-
ent colony, when raid columns were leaving the nest (that is, before
any new fight could have taken place). In total, we collected 763 min-
ors, 582 majors, and 502 intermediates (total *n* = 1847; Fig. 4).

**Velocity and mortality**

This experiment was conducted 20 times for each of the three catego-
ries (lost limb, termite clinging, and carried unharmed) in individual
raids, with an additional control test of healthy individuals (Fig. 1, A
and 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 on the basis
of the distance covered up to that incident. This allowed us to quantify
the handicap and mortality risk that each injury posed during the re-
turn journey (Fig. 2, A to E). If the ant stopped moving during the
return journey, most likely because of fatigue, the time was also stopped
and the velocity was calculated up to that point.

**Injury recovery**

To analyze the potential recovery of ants that lost an extremity, we
randomly cut off one leg on each side of a healthy ant (with scissors)
and 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 s
was measured. This experiment was repeated with the same individ-
ual 24 hours later and with healthy individuals as a control. These
experiments were conducted with laboratory colonies, so that we
could easily reproduce experimental parameters with regard to time
between experiments and nest conditions. We used acrylic pens to
mark individual ants, because previous observations showed no in-
dication of any lasting disturbance to the ants with this marking
method (fig. S2).

**Ethogram of rescue behavior**

Because there was no significant difference in the quantity of ants helped
at the hunting ground or on the return journey (Fig. 5B; Fisher’s exact
test, $P = 0.33; n = 20$), we carried out subsequent experiments (Fig. 5, A and C) during return journeys for easier reproducibility of trials. The experiments were repeated 20 times with at least five different colonies, with each return raid used for only one 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 behavioral reactions by the nestmates were recorded until the entirety of the column passed the study subject. The behavioral reactions of the helping ants consisted of five categories: (i) Ignored: contact with the study subject was less than 2 s; (ii) Investigated: the study subject was antennated for more than 2 s; (iii) Picked up: the study subject was fully lifted from the ground; (iv) Carried back: the study subject was carried back for at least 20 cm toward the direction of the nest; (v) Carried away: the study subject was removed from the return column in a direction that was not the one back to the nest, that is, away from the column. For graphical illustration and statistical analysis, we summarized behaviors (iii) and (iv) as rescue behavior and (iii) in combination with (v) as disposing of the study subject.

**Laboratory colonies**

Ten colonies were excavated and placed in artificial nests in the field station laboratory (colony size, 1373 ± 520 ants), consisting of a 20 × 20 × 10–cm large nest made of polyvinyl chloride connected to a 1 × 1–m arena. For raids, this arena was connected by a 10-m-long corridor to a second arena (1 × 1 m). The ground was covered with earth from the surrounding area. In the second arena, *M. 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 behavior on which we performed the injury recovery experiments. For further details on laboratory keeping, see Yusuf et al. (29).

**Pheromone and stridulatory communication**

To inhibit stridulation, we coated the stridulatory organ, located between the first and second tergites (22), with black acrylic paint. After the paint dried for 2 min, the experiment was conducted. To confirm that stridulation was truly inhibited, we triggered normal stridulation behavior by exposing the ant to CO2, as previously described by Hölldobler et al. (22). During this process, the sound was recorded with an external microphone (Speedlink SL-8703-BK, Jöllenbeck GmbH). To visualize the sound, a sonogram was created with the digital audio editor Audacity version 2.0.5.0 (Fig. S1A).

For the pheromone experiments, we dissected a gland and placed it on a glass surface, then pulled the thorax of the study subject three times over the burst gland reservoir (for smaller mandibular glands, 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 90 ng/ml. Subsequently, two drops (roughly 9 ng of the substance) were applied on a glass surface. The selected concentrations were similar to the quantities found in a mandibular gland [14 ng of DMDS and 5 ng of DMTS per gland in a major worker according to Longhurst et al. (17)] and a comparison of the mass spectrometer of the gland reservoir with our solution. After 30 s, 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 Comœ National Park (Côte d’Ivoire). The workers were then transported alive to the University of Würzburg (Germany) and killed with CO2 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 of pure pentane for 2 hours (two capit and six mandibular glands, respectively). These extracts were evaporated to a residue of approximately 100 μl. We used 1 μl extracts for GC-MS analyses, which were carried out on a gas chromatograph (6890) coupled to a mass selective detector (5975) from Agilent Technologies. The GC was equipped with a DB-5 capillary column (0.25 mm inside diameter × 30 m; film thickness, 0.25 μm; J&W Scientific). Helium was used as a carrier gas, with a constant flow of 1 ml/min. A temperature program from 60° to 300°C with 5°C/min and finally 10 min at 300°C was used, with data collection starting 2 min after injection. The mass spectra were recorded in the electron ionization mode, with an ionization voltage of 70 eV and a source temperature of 230°C.

The software ChemStation (Agilent Technologies) 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). Because of the very small quantities of DMDS and DMTS within the extracts, we used diagnostic ions and the retention time to confirm the identification.

**Rescue behavior model**

We designed an equilibrium model to quantify the possible benefits of rescue behavior to the colony. Benefit was expressed as the proportional increase in equilibrium worker number of a colony with rescue behavior compared to a colony that would not show this behavior. For the sake of argument, we chose 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 behavior is described by equation

$$\frac{dH}{dt} = b - (e_H + \mu_0)H$$  \hspace{1cm} (1a)

where $H$ is the number of noninjured (healthy) workers, $b$ is the rate at which new workers are added to the colony, $e_H$ is the rate at which workers are involved in injuring interactions with termites, and $\mu_0$ is the base mortality rate of workers.

For this colony, colony size (worker number) will settle into equilibrium

$$\dot{H} = \frac{b}{e_H + \mu_0}$$  \hspace{1cm} (1b)

In addition, a colony that manages to rescue a fraction $f$ (0 ≤ $f$ ≤ 1) of the workers injured in action will build a "pool" $J$ of workers that were injured in previous raids but were 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

$$\frac{dJ}{dt} = f e_H H - (\mu_f + \mu_0)J$$  \hspace{1cm} (2a)

where $f$ (0 ≤ $f$ ≤ 1) is the proportion of ants injured in combat that survive and $\mu_f$ is the added (future) mortality rate of injured compared...
to noninjured workers. For simplicity and on the basis of empirical observation, we conservatively assume that a second injury sustained in another raid would always be fatal. The equilibrium number of injured ants in a colony is thus

$$j = \frac{He_H}{\mu_j + \mu_0}$$

(2b)

The relative size of colonies with rescue behavior compared to a colony not showing this behavior, that is, the total loss of injured individuals ($f = 0$), is thus defined by

$$\frac{H + j}{H} = 1 + \frac{He_H}{\mu_j + \mu_0}$$

(3)

Quantification of model

The observed survivability of an injured ant not receiving help is 68% (Fig. 2A). Thus, $f = 0.68$ characterizes hypothetical colonies without rescue behavior, whereas in colonies where the behavior is present, $f = 1$, because all rescued ants were observed in later raids. All other parameters stay the same in both cases and were calculated as follows. Because we can only quantify the injury ratio in the colony for ants that lost an extremity, our value $e_H$ was defined as the percentage of lost limb injuries per raid (0.21-3) divided by the ratio of healthy ants in a colony (0.79-1373, $n = 10$ excavated colonies); therefore, $e_H = 0.0017$. We conservatively argue that the added mortality of a previously injured ant is the probability of getting injured again; therefore, $\mu_j = e_jp$ 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. In Eq. 1b, we were able to calculate $\mu_0 = 0.0076$. 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. 4) to our model prediction from Eq. 2b: 0.21. The good agreement of predicted and empirical values allowed us to reliably calculate the benefit of the rescue behavior by comparing the calculated colony size ($H + j$) of a colony with rescue behavior to one without. Our results indicate that the helping behavior 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. 1A). If extrapolated for all injuries, the benefit of the rescue behavior can be estimated to be 28.7%.

Statistical analysis

For statistical analysis and graphical illustration, we used the statistical software R version 3.1.2 (31) with the user interface RStudio version 0.98.501. We tested for deviations from the normal distribution with the Shapiro-Wilk 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 post hoc 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 analyze the ethogram data, a Fisher’s exact test with Bonferroni correction was used with a no-help control (0 of 20 helped) compared to our treatments. Median values mentioned in the text are followed by a median absolute deviation.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/3/4/e1602187/DC1

**fig. S1.** Illustration of a helping ant and different injury types, as shown in Fig. 1. fig. S2. Effect of stridulation on rescue behavior.

**table S1.** Statistical differences in injury-type frequency (Fig. 1A) and speed (Fig. 1B) in injured *M. analis* ants (Kruskal-Wallis test, followed by pairwise comparisons with Bonferroni-corrected Dunn’s test; $n = 20$ per test).

**table S2.** Statistical differences in running speed of individuals with different stages of injury, as shown in Fig. 3 (Kruskal-Wallis test, followed by Dunn’s test with Bonferroni correction; $n = 20$ per test).

**table S3.** Statistical differences in long-term injuries in the different castes, as shown in Fig. 4 (ANOVA test, followed by Tukey post hoc test; $n = 20$ per test).

**table S4.** Statistical differences in significance of rescue behavior compared to behavior of healthy individuals, as shown in Fig. 4 (A to D) and fig. S2 [Fisher’s exact tests for count data between treatment healthy (no help) and the other categories with Bonferroni correction].

**fig S1.** Movie S1. An injured ant receiving help by its nestmates.

**fig S2.** Effect of stridulation on rescue behavior.

**Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/3/4/e1602187/DC1**

**REFERENCES AND NOTES**


Acknowledgments: We thank V. Frank, B. Fiala, S. Leonhardt, K. Stein, and the anonymous referees for critical discussions and reading the manuscript. We thank the Comoé National Park Research Station for the use of their facilities for the field research and the park management of Office Ivoirien des Parcs et Réserves for facilitating field research in the park. Funding: O.M. and T.H. are funded by the German Research Foundation (DFG), Collaborative Research Center (grant number SFB 1047, “Insect Timing,” Project C6). E.T.F. was supported by a grant of the German Excellence Initiative to the Graduate School of Life Sciences, University of Würzburg (grant number GSC106/3). This publication was funded by DFG and the University of Würzburg in the funding programme Open Access Publishing.

Author contributions: E.T.F. and K.E.L. designed the study and collected and analyzed the field data. E.T.F. wrote the paper. T.S. performed MS analysis and contributed to the study design. J.S. conducted part of the field experiments. T.H. and O.M. developed the model. K.E.L. initiated and supervised the study. All authors discussed the results and commented on the manuscript. Competing interests: The authors declare that they have no competing interests. Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Submitted 9 September 2016
Accepted 16 February 2017
Published 12 April 2017
10.1126/sciadv.1602187

Saving the injured: Rescue behavior in the termite-hunting ant *Megaponera analis*

Erik Thomas Frank, Thomas Schmitt, Thomas Hovestadt, Oliver Mitesser, Jonas Stiegler and Karl Eduard Linsenmair

*Sci Adv* 3 (4), e1602187.
DOI: 10.1126/sciadv.1602187

ARTICLE TOOLS  http://advances.sciencemag.org/content/3/4/e1602187

SUPPLEMENTARY MATERIALS  http://advances.sciencemag.org/content/suppl/2017/04/10/3.4.e1602187.DC1

REFERENCES  This article cites 28 articles, 3 of which you can access for free  http://advances.sciencemag.org/content/3/4/e1602187#BIBL

PERMISSIONS  http://www.sciencemag.org/help/reprints-and-permissions